The Clinical Society of the New York Diabetes Association, an Affiliate of the American Diabetes Association, Third Symposium Day on Diabetes Mellitus

The Pituitary-Adrenal System and Diabetes Mellitus

ON behalf of the Association, I want to welcome you to this Third Annual Symposium Day of the New York Diabetes Association.

Three years ago, when we first decided to plan such a symposium, it was an experiment which we hoped would succeed in bringing to the physicians and research workers of this area, news about research in diabetes. At that time the Association had in its Research Fund the sum of $2,000, a gift of Mr. Felix Morgenstern, and the problem was how to put this money to the most productive use. It could have been employed to finance a single research project, but after much discussion it was decided that the greatest return on that money could be achieved by organizing a symposium day to disseminate and publish the results of the research of the year.

That symposium was far more successful than we had anticipated and brought to the physicians, students, and research workers of New York very stimulating and fertilizing ideas, and so it was decided to repeat the symposium day last year.

The papers of the first symposium were published in a volume through the kindness of the National Vitamin Foundation, and they had wide distribution.

Last year's symposium was even more successful, and as for this year, I think you can all understand how it has gotten out of hand. The quality of the workers represented on the program and the papers to be presented have succeeded in attracting many more applications for attendance than we have room for in this hall.

On behalf of the Association, I want to thank the distinguished workers who have consented to take part in this program. I also want to thank Dr. C. P. Rhoads and the administration of the Memorial Hospital and the Sloan-Kettering Institute for their great kindness in inviting us to use this hall.

Just one word for those who would like to know something about the Association itself. The Clinical Society of the New York Diabetes Association is its professional arm and is responsible for all its professional activities. Membership in the Clinical Society is open to all physicians, research workers, and teachers in this field. The dues are nominal. The purpose is to furnish a common meeting ground for people interested in this type of work, to help all workers in their efforts to learn more about diabetes, and to promote the welfare of diabetics. If you are interested, leave your name and address at the door or write to the Association.

This program was arranged under the leadership of Dr. George E. Anderson as Chairman of the Clinical Society, 1955-56.

HENRY E. MARKS, M.D., *President*
New York Diabetes Association

Presented at the Auditorium, Memorial Center for Cancer and Allied Diseases, 410 East 68th Street, New York City, on Thursday, Oct. 27, 1955.

Metabolism of C^{14}-Labeled Glucose in Diabetes

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A single unifying explanation has been sought for the many metabolic defects which have been observed in studies of experimental diabetes. Authors who have found derangements in fat, carbohydrate and protein metabolism have been inclined to attribute the observed disorders to a single biochemical disturbance. In figure 1 an abbreviated metabolic map is shown. The blocks indicate aspects of metabolism which have been demonstrated to be altered in experimental diabetes. The single-block hypothesis would attribute all or most of these defects to a relative inability of cells from diabetic tissue to form glucose-6-PO_4† (the so-called "primary lesion"). For example, Baker et al. have attempted to explain the lessened ability of liver slices from alloxan diabetic rats to form labeled fatty acids from radioactive acetate (see figure 1) as a secondary effect of an initial block in the ability of these livers to phosphorylate glucose.[3]

Although the one-block hypothesis may not be sufficient to explain all of the lesions associated with a relative insulin deficiency,[1] some authorities have favored the hypothesis, at least in modified form.[2, 4] The present paper, which is not intended as a comprehensive review, is concerned with several experiments by other workers and by the author which have a bearing on the validity of the one-block hypothesis. The author's presentation will be oriented around the following thesis:

The one-block hypothesis, as indicated above, requires that the many metabolic defects characteristic of tissues from diabetics may be described as secondary effects of a *primary* depressed rate of glucose phosphorylation. However, when a comparison is made between rates of initial glucose phosphorylation in tissues from *hyperglycemic* diabetics and euglycemic normals, the experimental data do not yet allow a conclusion to be drawn regarding the existence of this primary block.

In 1937, Soskin and Levine presented a paper which explained how evidence could indicate the existence of a block in diabetic glucose utilization when, in fact, glucose utilization* might be proceeding at as rapid a rate in the diabetic as in the normal.[5] Before reviewing some pertinent features of this classic work, it might be well to point out that their paper serves not only as an historical reference. It presents a hypothesis which warrants careful consideration in all work dealing with the rate of glucose utilization in diabetic as compared to normal tissue. Its relation to the one-block hypothesis is of special importance, for it suggests that hyperglycemia alone may compensate fully for a potentially diminished rate of glucose utilization in the diabetic. If this is so, defects in other areas of metabolism could hardly be considered as *secondary* to a lessened rate of carbohydrate uptake by tissues.

Using eviscerated "normal" and eviscerated pancreatectomized dogs, Soskin and Levine were able to bring the blood sugar concentration of either preparation to almost any desired level by means of a constant infusion of glucose. Since the animals had no livers or kidneys,

†Excellent reviews on the mechanism of action of insulin by Stadie[1] and by Levine and Goldstein[2] have been published recently. Included in these reviews were experiments which have attempted to pinpoint the exact site of an early defect in glucose utilization (transfer of free glucose into the cell, phosphorylation of glucose within the cell, and, in the case of liver, enzymatic dephosphorylation of glucose-6-PO_4). A defect in the ability of a cell to form glucose-6-PO_4 may involve any or all of these processes. This problem will not be discussed further in this paper.

Presented in part at the Symposium on The Pituitary-Adrenal System and Diabetes Mellitus, presented by the New York Diabetes Association, Oct. 27, 1955.

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*Glucose utilization, as defined by Soskin and Levine, referred to net glucose removal from the extracellular fluid *and from muscle glycogen* by all processes other than urinary excretion and lactic acid formation. In subsequent discussion of other papers, this author will refer to "the rate of glucose utilization" as the rate of removal of glucose from the extracellular fluids by all processes other than urinary excretion. Furthermore, it will be assumed that this rate of utilization is a measure of the relative rate of conversion of extracellular glucose to intracellular glucose-6-PO_4. When the rate of formation of some "end product" of glucose metabolism such as CO_2 or glycogen is used to develop the author's thesis, it should be remembered that such a rate need not necessarily be an index of the relative rate of *utilization* (as defined above). On the other hand, a depressed rate of CO_2 or glycogen formation from glucose may be considered to be *consistent with* the existence of a defect in the region of initial glucose phosphorylation.

the only source of blood glucose was, of course, that of the infusion fluid. Thus by determining the rate at which glucose had been introduced into the blood stream, along with some other variables, a measure of the rate of glucose utilization* could be obtained as a function of blood sugar concentration as shown in figure 2. Four relationships which the curves of this figure bring out and which are pertinent to the following discussion are:

First, at a given blood sugar level up to at least 500 mg. per cent, the normal utilized glucose at a faster rate than the diabetic.

Second, however, at a blood sugar concentration of 600 mg. per cent, the diabetic dog oxidized glucose at a greater rate than did the normal at 100 mg. per cent sugar concentration.

Third, as the blood sugar concentration is raised in the pancreatectomized dog from 120 to 600 mg. per cent, an increase of approximately five-fold in the rate of utilization is obtained.

Finally, the curves converge, a fact which indicates that there is no effect of insulin-lack at very high blood sugar concentrations.

Several experiments have been cited as being contradictory to the Soskin and Levine hypothesis. Of these only the work of Wick and Drury seem to this author to be a valid challenge to the work of Soskin and Levine. Wick and Drury worked with eviscerated, pancreatectomized rabbits.[6] In order to study the effect of blood glucose concentration upon the rate of utilization, they used some aspects of the constant infusion technic which was described above as part of Soskin and Levine's approach. (In addition, they measured the rate of radioactive CO_2 formation from glucose-C^{14}; however, the measurement of rate of utilization seems more pertinent to the present discussion.) Wick and Drury found that, in the absence of any added insulin, increasing the blood sugar concentration from 163 to 600 mg. per cent only raised the rate of utilization by about 16 per cent. A comparable rise in blood sugar concentration in Soskin and Levine's experiments, you will recall, produced about a 400 per cent rise in rate of utilization.

Two other experiments which have been cited in a review[7] (not in the original papers) as further evidence against the Soskin and Levine hypothesis are those of

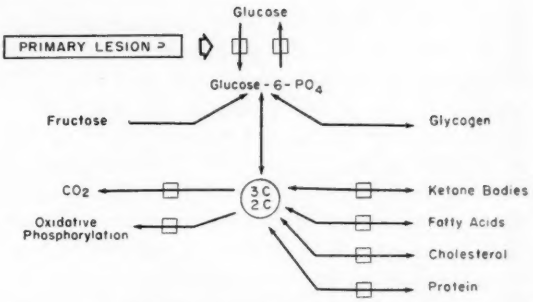


FIG. 1. An abbreviated metabolic map. The blocks indicate areas of metabolism for which there is some evidence of defect in experimental diabetes.

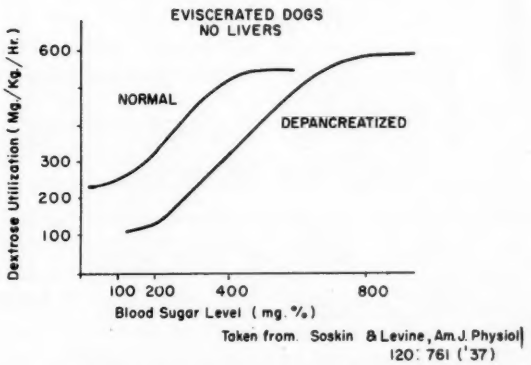


FIG. 2. The effect of increasing blood sugar concentration upon dextrose utilization in "normal" and depancreatized dogs.[5] See footnote in text for the definition of utilization employed by Soskin and Levine.

Stadie et al.[8] and of Stetten et al.[9] Neither of these papers seems to this author to be inconsistent with the work of Soskin and Levine.

Stadie and his co-workers incubated, with varying concentrations of glucose, diaphragms which either had or had not been pretreated with insulin; they then measured the extent of glycogen synthesis in ninety minutes (figure 3). As can be seen from the data an insulin effect *is* apparent even at concentrations of 3 per cent (3,000 mg. per cent), and in this respect the data might be considered to differ from those of Soskin and Levine. On the other hand, it should be noted that consistent with Soskin and Levine's experiments, the extent of glycogen synthesis is approximately as great without insulin at a glucose concentration of 400 mg. per cent as it is with insulin at a concentration of 90 mg. per cent. In any case, the failure of the curves to converge at a reasonably high blood sugar concentration should not be taken as evidence against the more important features of the

*The data of Soskin and Levine have been recalculated, expressing utilization in terms of simple, nonexcretory glucose removal from the total extracellular fluid. Relationships very similar to those seen in figure 2 are obtained; however, the "maximum" height of each curve is about one-third lower, while the slopes of the relatively linear segment remain about the same.

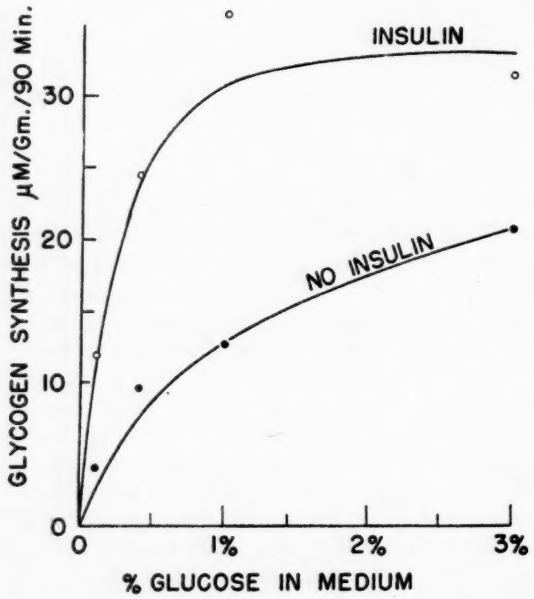


FIG. 3. The effect of glucose concentration in the medium upon the capacity of normal diaphragm muscle to synthesize glycogen from glucose in vitro. The diaphragms were pretreated for a short period of time with one unit of insulin in the case of the upper curve. Taken from Stadie et al.[8]

Soskin and Levine curves.*

Another experiment which has been cited by Haugaard and Marsh[7] as evidence against the Soskin and Levine hypothesis is that of Stetten et al.[9] In Stetten's experiments hyperglycemia (for the sake of producing glycosuria) was induced in normal rats by the constant infusion of a high concentration of glucose. Glucose at a lesser rate of infusion was injected into the glycosuric diabetic rats. Comparison between the incorporation of radioactive glucose into CO_2 by *hyperglycemic* normal and diabetic rats was then made. The authors concluded that CO_2 formation from glucose was markedly depressed in the diabetic rats compared to the hyperglycemic normals. However, as Strisower pointed out,[13] these studies do not appear to be relevant to the question of whether or not the diabetic at a hyperglycemic level can oxidize glucose as rapidly as a normal subject having a lower blood sugar concentration.

Thus far in our discussion, the one-block hypothesis has been defined; support for such a hypothesis requires that a depressed rate of glucose utilization (uptake by tissues) be demonstrable in the *hyperglycemic* diabetic as compared to the *euglycemic* normal. The work of Soskin and Levine in eviscerated dogs suggests that no such defect may exist if comparisons are made at the different blood sugar concentrations typical of the untreated diabetic and the normal. On the other hand, the work of Wick and Drury with eviscerated, pancreatectomized rabbits failed to demonstrate any marked effect of raising the blood glucose concentration upon the glucose utilization rate.

Is there, then, some noncontroversial basis for the concept that glucose is not being utilized adequately by tissues of the hyperglycemic diabetic? Carbon-14-labeled isotopes, which are of special value in experiments dealing with isolated liver and in in vivo studies where the liver is functional, have been used in an effort to clarify this problem. Isotopic glucose, for example, has enabled workers to study the utilization of glucose in intact subjects, where hepatic glucogenesis may keep pace with glucose utilization so that no net change of blood glucose occurs.

Perhaps the most oft-quoted references of the isotopic era which are cited as evidence for the existence of depressed glucose utilization* in the diabetic liver are those of Chernick, Chaikoff and co-workers.[14, 15] This work has also served as a basis for the conclusions drawn in a paper by Baker et al.,[3] which, as was mentioned earlier, is part of the evidence considered to favor the so-called one-block hypothesis. An attempt will not be made to point out how, to my mind, the Chernick-Chaikoff paper has been misinterpreted—not by the authors in their original paper, but by those of us who at times have forgotten the data while remembering their rather simple conclusions: Glucose oxidation is depressed in diabetic liver, the site of the lesion being, probably, hexokinase.

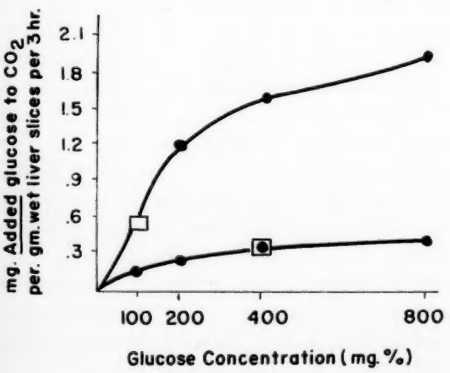
Briefly, their experimental approach was as follows: Liver slices were prepared as rapidly as possible from fed, untreated alloxan diabetic and normal rats. The liver slices were incubated in a buffer solution contain-

*If in vitro experiments using diaphragm muscle may be compared meaningfully with the experiments of Soskin and Levine, it might be best to cite experiments in which the rate of glucose utilization (disappearance) is measured rather than the rate of glycogen formation. Most studies using isolated diaphragms from normal and diabetic animals have shown an increased uptake of glucose as a result of raising the concentration of glucose in the incubation medium.[10, 11, 12]

*The attempt has been made thus far to confine our discussion to studies which measure glucose *utilization*. The experiments of Chernick et al. may be grouped in this category in only a limited sense. By using isotopic tracers such as fructose-C^{14}, in addition to radioactive glucose, these workers were able to refer observed differences in glucose *oxidation* to relative differences in the rates of initial phosphorylation of glucose.

ing glucose at various concentrations. The glucose was labeled with radioactive carbon by means of which the incorporation of glucose carbon into other compounds such as carbon dioxide, could be traced. From the radioactivity which is incorporated into the CO_2 one may calculate the mg. of *added* glucose which are oxidized to CO_2 in a given time period since the activity per mg. of added glucose is known. However, as the authors have pointed out, one cannot state from such data what the *true* rates of oxidation are, since a considerable and quantitatively undetermined dilution of the isotope takes place during the incubation, primarily through glycogenolysis and glucogenesis. As a result, one is limited in the conclusions which may be drawn from such data. For example, if one assumes that the dilution of glucose at any given initial glucose concentration is the same in diabetic and normal liver slices, for which assumption there was some evidence in Chernick's experiments, then the extent of incorporation of C^{14} into CO_2 would serve as a valid index of the relative rates of oxidation. On the other hand, it is quite improbable that the relative dilution of isotope will be the same at two *different* initial glucose concentrations; therefore as Chernick and coworkers were careful to point out, it was not possible to evaluate from this type of study whether or not glucose oxidation is increased at higher concentrations of glucose ([14], p. 531).

This point may be illustrated by means of figure 4 which summarizes some of the work which Chernick carried out in Dr. Chaikoff's laboratory.[16] Here it may be seen that the extent of incorporation of C^{14} from glucose



Taken from: Chernick, Metabolic Fuels, Brookhaven ('52)

FIG. 4. Glucose oxidation to CO_2 in liver slices from normal (upper curve) and alloxan diabetic rats (lower curve) at different glucose concentrations in the incubation medium.[16]

into CO_2 is depressed in the liver of the diabetic (lower curve) at every single concentration of glucose tested. This is not to say that the data demonstrate glucose oxidation is greater in the normal liver at 100 mg. per cent than in that of the diabetic at 400 mg. per cent. I make this point here since the curves of figure 4, at casual inspection, suggest the latter statement and such an inference was probably not intended by the author. (The point representing the normal glucose oxidation rate at 100 mg. per cent glucose concentration was omitted by Chernick. A cursory examination of the published data would indicate that the curve at this point appears to be erroneously high relative to the diabetic rate at 400 mg. per cent glucose concentration. However, data at the 100 mg. per cent level are very limited since a higher initial glucose concentration, which lessens errors due to dilution, was used in most of these experiments.)

It should be obvious then that before some answer is obtained to the basic question regarding the ability of diabetic liver to oxidize glucose at an adequate rate under conditions of hyperglycemia, a more complicated analysis, one which takes into account dilution factors, must be undertaken. A study which considers the dilution of radioactive glucose during the incubation of liver slices has been reported recently by Renold et al.[17] This work confirms the less detailed experiments of Chernick and Chaikoff which indicated that *at the same concentration of glucose,* the initial phosphorylation of glucose seems to be depressed in diabetic livers; however, no attempt was made by Renold et al. to compare rates of glucose utilization at various sugar concentrations.

I would like to illustrate, now, two instances in which the work of Chernick and Chaikoff was prematurely extrapolated. The first of these occurred when certain authors were unable to reconcile two papers which originated from Dr. Chaikoff's laboratory, one, by Chernick and Chaikoff stating that glucose oxidation was depressed in diabetic liver slices,[15] and the other by Feller et al. that glucose oxidation was normal in the intact, *hyperglycemic,* alloxan diabetic rat.[18] Actually the two papers do not necessarily conflict.[13] As we saw, the paper concerned with diabetic liver slices had not shown that the rate of glucose oxidation at high glucose concentrations in the diabetic was less than that at lesser concentrations in the normal liver. No prediction was to be made from the in vitro data concerning the important question: "Can the diabetic liver, exposed in vivo to hyperglycemia in the absence of insulin, utilize as much glucose as that of the normal liver at normal blood sugar concentrations?" (Also as Feller et al. pointed out,[18] even if the rate of glucose utilization by liver *were* depressed in the intact

animals, such a decrease might well be masked by an active peripheral oxidation.)

A second instance of incorrect extrapolation is that of a paper with which the author was associated.[3] In designing these experiments, the authors reasoned as follows: (1) Chernick and Chaikoff have shown that glucose utilization is depressed in diabetic liver; (2) in liver slices from hyperglycemic diabetic rats which had been fed glucose, fatty acid "synthesis" from acetate-C^{14} is depressed; (3) perhaps the defect in the synthetic process is only a secondary effect of the liver's not having utilized glucose sufficiently before the liver was removed for study. . . .

The error in this logic arises from the fact that it was not fully recalled, by this author at least, that the first statement in the above logic was only partly true. Chernick and Chaikoff may have shown that, *at a given glucose concentration,* glucose utilization in liver slices from diabetic rats was depressed compared to that of the normal. However, they had not demonstrated that glucose utilization in the diabetic's liver was depressed under conditions of hyperglycemia compared to the normal liver under conditions of euglycemia. Since it is still to be ascertained whether or not hyperglycemia can compensate for the potential defect in glucose utilization which is found in the liver of the alloxan diabetic rat, final interpretation of the results of Baker et al. should be postponed until the effect of hyperglycemia upon glucose utilization in the liver of the diabetic has been studied further.

So far, a picture of confusion has been painted with respect to the compensating effect of hyperglycemia upon glucose utilization in both extrahepatic and hepatic tissues of the diabetic. Let us consider some experimental attempts to study this problem in the intact subject.

It is felt that one of the most fruitful approaches to the problem will be an expansion of that used by several workers in Dr. Chaikoff's laboratory[18, 19] in which a single tracer dose of glucose C^{14} is administered intravenously to diabetic and normal animals without altering markedly their usual blood sugar levels. An attempt to analyze the rate of change of isotope concentration in various pools may then be made under relatively nonartificial conditions. This approach, however, is fraught with technical and theoretical difficulties. An example of these difficulties may be illustrated by some of our recent work using this technic with intact normal and diabetic humans and with normal and alloxan diabetic rats. It was desired to establish what were the over-all average rates of glucose utilization and oxidation to carbon dioxide in these studies. Previously estimates had been made based upon a one-compartment model,[18, 19] and then a two-compartment model.[20] The relative simplicity of these basic models is illustrated in figure 5. Here, it will be seen the assumption is made that glucose is oxidized to CO_2 by way of pools of intermediates which are negligible in size relative to that of the glucose and the bicarbonate pools of the body; hence, not indicated in the figure. Dilution of labeled glucose through hepatic glucogenesis is taken into account in each case as indicated by the arrow to the left of each model. However, only in the lower system is it taken into account that there is a considerable hold-up of C^{14} in the sizeable body bicarbonate pool prior to the expiration of glucose-derived $C^{14}O_2$. If serial measurements are made of the specific activity of blood glucose and respiratory CO_2, curves are obtained of the following sort, taken from Shreeve et al.[22] In figure 6, the data obtained from three labile ketotic diabetic humans are compared with those, on the left, of a composite of data from four normal subjects. In the case of the diabetics, the lower specific activities of the glucose and CO_2 curves, the lesser slope of C^{14} glucose decay, and the later time of maximum CO_2 specific activity are all, in part at least, due to the greater dilution of the injected radioactive glucose by the high blood sugar concentration of the untreated diabetics. The fraction of the CO_2 which is derived from glucose is given by the ratio of the CO_2 glucose specific activity at t_{max}. The broken curve is a mathematically derived reference curve, the significance of which need not be discussed at this time.[20, 22] In figure 7, this fraction, expressed as percentage, is indicated for each of four normal humans, three nonketotic diabetics, and five ketotic diabetics. It may be seen that, in the ketotic subjects, the oxidation of glucose tends to be depressed.

The rate of nonexcretory glucose utilization in the normal, ketotic diabetic and nonketotic diabetic subjects averaged approximately 0.08 gm./kg./hr. for each group. Since the spread of values was large (.03-.14) and the groups small in number, it may be premature to speculate from these data. However, in terms of the Soskin and Levine hypothesis, these findings have been interpreted to indicate that hyperglycemia may compensate for a "relative insulin deficiency" in the human diabetic. Such compensation need not be complete since there was a definite tendency toward a depressed rate of glucose oxidation in the ketotic subject.

It is of interest that, in the experiments of Feller et al.,[19] the rate of utilization in the hyperglycemic diabetic dog, measured in the same manner as in the human studies, was not significantly different from that of the euglycemic normal (table 1). However, the validity of

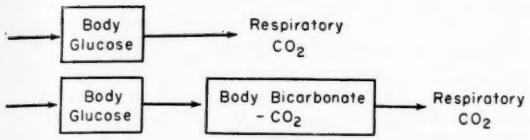


FIG. 5. One pool and two pool models previously assumed to describe the approximate manner of glucose oxidation in vivo.

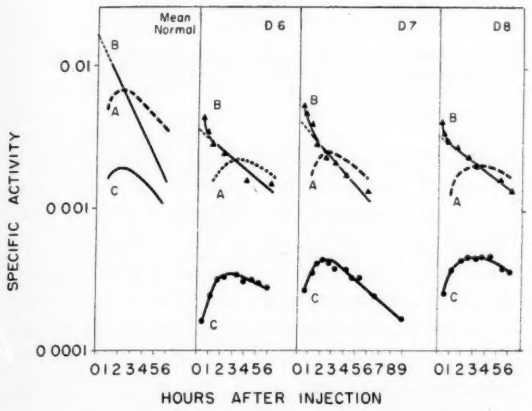


FIG. 6. Curves of specific activity for plasma glucose (B) and expired CO_2 (C) obtained in ketotic, labile diabetic human subjects are shown along with composite normal curves. The uppermost reference curve (A) is explained in [20, 22]. Specific Activity is expressed as per cent of administered C^{14} per mg. of either glucose or CO_2 carbon. The scale on the ordinate is logarithmic. Taken from [22].

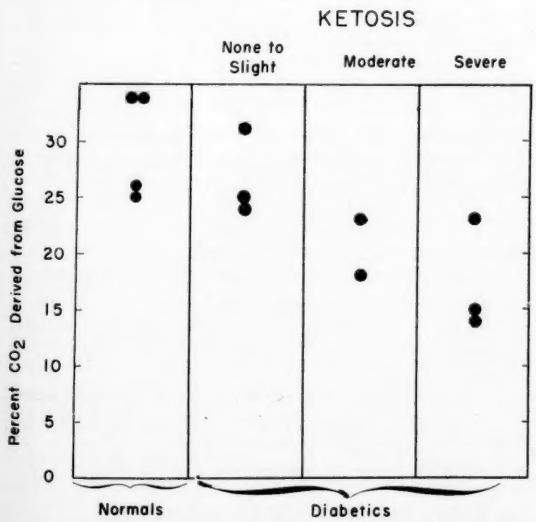


FIG. 7. Glucose oxidation to CO_2 in normal and diabetic human subjects. The units of the ordinate may be used as a fair index, usually, of the relative rate of glucose oxidation. Taken from [22].

these determinations is dependent upon an accurate estimation of the glucose pool size. The latter values have been questioned by workers from the same laboratory.[21] The oxidation of glucose in dogs,[19] when recalculated,[20] tends to be depressed in the diabetic compared to the normal dog.

TABLE 1

Rate of glucose utilization in dogs as studied with glucose-C^{14}

Dog	State	Nonexcretory rate of utilization gm./kg./hr.
D	Normal	.32
	Diabetic	.32
A	Normal	.38
	Diabetic	.36
C	Diabetic (not studied in normal state)	.19

Calculated from the data of Feller et al.[9]

More recently it has been found necessary in working with rats to apply a six-compartment model such as that shown in figure 8. The body glucose and bicarbonate pools are subdivided into plasma and extra-plasma compartments, and the phenomenon known as recycling is taken into account. This model requires a very complex analysis of the experimental data; however, there is reason to think that more accurate approximations of the rates of glucose utilization and oxidation in vivo may be attained by using such an approach.

We are currently attempting to analyze data obtained with intact alloxan diabetic and normal rats to determine whether our earlier conclusions based on a simpler model, were justified. Previously in a preliminary communication, it was suggested by us that, in hyperglycemia, the 16-24 hour fasted alloxan diabetic rat can sustain as rapid a rate of glucose oxidation as is carried out by similarly fasted euglycemic normal rats.[23]

In summary, an attempt has been made here to evaluate the present status of the literature with regard to some aspects of the problem of glucose utilization in the diabetic. In 1937 Soskin and Levine thought that they had at long last resolved a controversy which had been proceeding at the expense of much wasted motion and emotion. Unfortunately, eighteen years later, in 1955, the problem does not yet seem to have been resolved. The principal reasons for this failure have been recognized: With few exceptions, experiments involving the use of tracers have simply not been designed to test the question of whether or not glucose utilization, which seems to be depressed at any given blood sugar concentration in tissues of the diabetic, may proceed at an adequate rate in the presence of a high concentration of

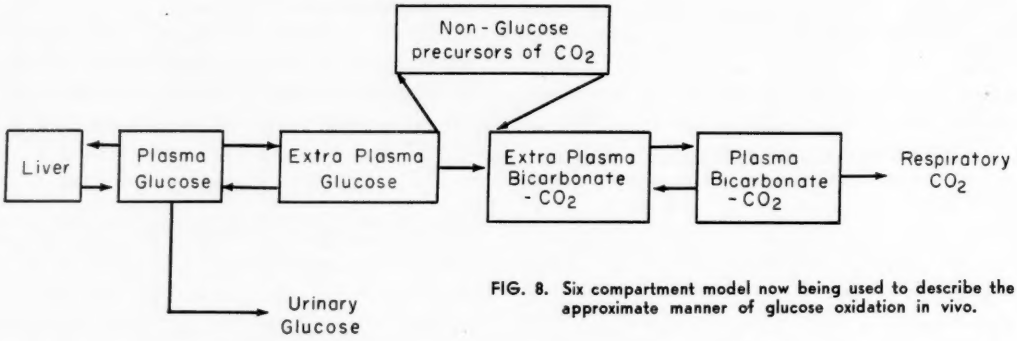


FIG. 8. Six compartment model now being used to describe the approximate manner of glucose oxidation in vivo.

glucose. Those experiments which, by design, do allow testing of the Soskin and Levine hypothesis, in part, confirm and, in part, refute the hypothesis. Considerably more experimental work will be required before it will be established what the actual and relative rates of glucose utilization are in tissues of the intact diabetic. Present-day explanations of defects in lipid and protein metabolism based upon a single *primary* defect in carbohydrate utilization should be regarded as mere conjecture and not as well-developed theories founded upon sound experimental bases.

ACKNOWLEDGMENT

I would like to thank Drs. S. S. Chernick, S. Soskin, and W. C. Stadie, the Editors of *The American Journal of Physiology* and of *The Journal of Biological Chemistry*, as well as the Technical Information Division of Brookhaven National Laboratory for granting their permission to use figures 2, 3, and 4 of this paper.

SUMMARIO IN INTERLINGUA

Metabolismo de Glucosa Etiquettate a C-14 in Diabete

Esseva interprendite le tentativa de evalutar le stato presente del litteratura in re certe aspectos del problema del utilisation de glucosa in diabeticos. In 1937 Soskin e Levine credeva que illes habeva finalmente resolvite le controversia que habeva costate tante motiones e emotiones perdite. Infelicemente, 18 annos plus tarde, in 1955, il pare que le problema es non ancora resolvite. Le principal rationes pro iste manco de progresso es nunc cognoscite. Con pauc exceptiones, experimentos involvente le uso de traciatores ha simplemente non essite planate pro clarificar le question de si o non le utilisation de glucosa (que pare esser deprimite a omne date concentration de sucro in le histos del diabetico) pote proceder a un tempo adequate in le presentia de un alte concentration de glucosa. Le experimentos in que le plano fundamental esseva concipite de maniera a permitter le probation del hypothese de Soskin e Levine confirma lo in parte sed etiam refuta lo in parte. Un quantitate considerabile de experimentation additional va esser necessari ante que on pote establir le ver e le relative valores del utilisation de glucosa in le histos del diabetico intacte. Le currente explicationes de defectos in le metabolismo lipidic e proteinic, basate super un sol defecto *primari* in le utilisation de hydratos de carbon, deberea esser considerate como conjecturas e non como ben disveloppate theorias a solide bases experimental.

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DISCUSSION

RACHMIEL LEVINE, M.D. (*Chicago*): I should like to point out, regarding Dr. Baker's discussion of the relationship between blood level or that of sugar in the medium, and glucose utilization, that the original paper, in 1937 (Soskin and Levine) refers only to glucose utilization as defined by the disappearance of glucose as such or in the form of glycogen and lactic acid in the eviscerated nephrectomized dog. It does not refer at all to oxidation, since we did not measure it. The direct relationship of level of glucose to its utilization was found by Lundsgaard in the perfused hind limb and by other workers using the rat diaphragm (Gemmill, Villee and Hastings). This of course has nothing to do with oxidation in the strict sense of the word. Specifically, we had no knowledge at all of whether that relationship held for liver.

The rates of glucose utilization of the tissues of the eviscerated dog we have since found, are lower than they are in the presence of the liver, which makes for another complication.

ROBERT STEELE, M.D.,* (*Brookhaven National Laboratory*): I want to talk about the glucose pool sizes which Dr. Baker has calculated for the human, which I think are too high, and about his calculated glucose oxidation rates, which I think are too low.

Our own group, which consists of Drs. de Bodo, Wall, Altszuler and myself, has measured the body glucose pool in dogs. Instead of the single injection technic as described here today, we prefer to give a constant intravenous infusion of C^{14} glucose subsequent to an intravenous "priming" dose. When the plasma glucose specific activity is kept high by the constant infusion the effects of recycling are minimized. Recycling refers to the release into the plasma of labeled glucose resynthesized by the liver from C^{14} fragments (e.g., lactate) derived from the administered labeled glucose. In Dr. Baker's normal humans (his figure 6) the plasma glucose specific activity at five hours has about one-fifth its value at one hour. It is easy for a small amount of recycled C^{14} to raise the observed plasma glucose specific activities at the later time intervals by a considerable amount (percentage-wise) in this kind of an experiment. The effect of this is to cause the straight line in Dr. Baker's graph to be displaced counterclockwise around his earliest observed point. The line extrapolated back to zero time gives a value for initial specific activity which is too low so the calculated size of the glucose pool is too high.

Besides this theoretical reason for believing that Dr. Baker's glucose pool sizes are too high, there have been a number of measurements of tissue glucose concentration which may be used to get an idea of how big the body glucose pool should be. The amount of true glucose in tissues is so small that even if it were considered to be all extracellular the calculated interstitial fluid glucose concentration in the normal animal under ordinary conditions would be about the same as the plasma glucose concentration. Dr. Baker's calculated glucose pool size for the normal human, however, requires a space which is bigger than the inulin space. In the normal dog by the priming dose-constant infusion technic we get a glucose space which equals the inulin space.

Now with regard to glucose oxidation, Dr. Baker has used a relatively linear portion of the respiratory CO_2 specific activity (equivalent to blood HCO_3 specific activity) curve after $NaHC^{14}O_3$ injection to represent a "half-time" for the body HCO_3 pool. Figure 1 represents the HCO_3 pool of the cat, and the kind of result ob-

* Part of the work discussed was done at Brookhaven National Laboratory under the auspices of the Atomic Energy Commission.

tained by Dr. Baker's treatment of the data is illustrated there by the dotted line corresponding to a half-time of thirty minutes.

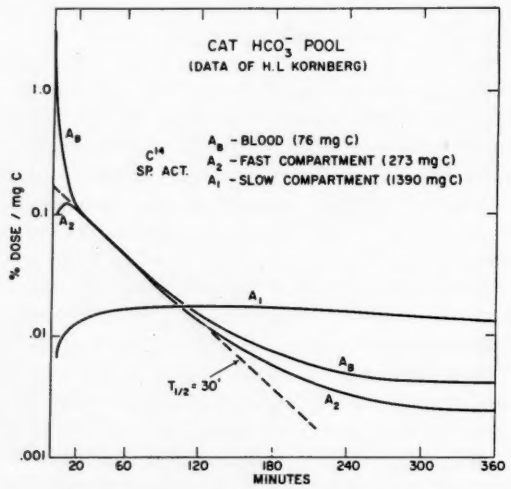

FIG. 1. Specific activities of the compartments of the cat HCO_3 pool following an intravenous injection of $NaHC^{14}O_3$.

The basic data for figure 1 were published by Kornberg, Davies and Krebs. I have shown that the shape of their $HC^{14}O_3$ retention curve requires the presence in the cat HCO_3 pool of the three compartments shown in figure 1 (*Biochem. J.* 60:447, 1955). It is apparent that the dotted line of figure 1 is characteristic of the C^{14} content of only a part of the HCO_3 pool and of that part for only a limited period.

A neglected part of the pool which is of great importance is the large slow compartment which consists mostly of bone carbonate. Some of the $C^{14}O_2$ from C^{14} glucose oxidation becomes hidden in this large slow compartment. Thus more C^{14} glucose has to be oxidized to yield a given blood $HC^{14}O_3$ concentration than is calculated by the 30 minute half-time approximation. This is illustrated by the following example.

Figure 2 shows a hypothetical constant infusion experiment in the cat. Here all of the CO_2 entering the fast compartment of the HCO_3 is thought of as having the same specific activity as the plasma glucose. This is the "100% CO_2" curve in the sense meant by Dr. Baker in his work in the human. At the end of five hours this "100% CO_2" curve lies at 0.78 whereas Dr. Baker's "100% CO_2" curve based on a 30 minute half-time for the HCO_3 pool would lie at 0.999. Most people have regarded this length of time of constant infusion as enough to produce a "uniform" specific activity in the HCO_3 pool and their "100% CO_2" curve would lie at 1.00. We have ourselves reported such experiments in the dog and Feller and his co-workers have reported others in the dog which Dr. Baker uses for comparison with his values in the human. All of these including Dr. Baker's present report probably include the same error—too low a value for glucose oxidation.

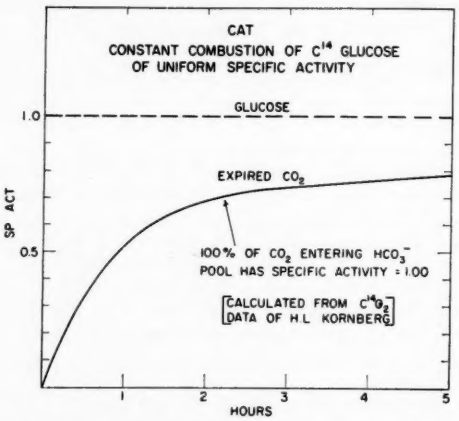

FIG. 2. Reference curve for calculating the fraction of respiratory CO_2 derived from plasma glucose in a C^{14} glucose constant infusion experiment in the cat.

Also in comparing normal glucose oxidation with diabetic, I think it is very doubtful whether the HCO_3 pool of the patient with diabetic ketosis acts the same as the HCO_3 pool of the normal individual. Test doses of $HC^{14}O_3$ in diabetics in ketosis would settle this point.

In conclusion I wish to remark that experimentation of this kind is in the pioneering phase and that Dr. Baker and the other members of the group at Cleveland have made a significant contribution by extending the work to humans and human diabetics. There is no doubt that in the diabetic state glucose is burned to CO_2. As to whether significantly less glucose is burned by the diabetic at his prevailing level of hyperglycemia, this question cannot be answered until the details of interpretation of the experiments with C^{14} glucose are better worked out.

DR. BAKER: I would agree with Dr. Steele's criticisms. They are very valid. We are taking them into account in our more recent work. In rat data the complications are so great that one cannot at all use such a simple model as we used in humans. But I have another slide, which I won't show now, with more extensive data in humans which would indicate that the "linear portion" of the curve is really quite linear between one hour and six hours. In rats the curve would not be truly linear for more than, I think, a half-hour or an hour.

Experimental Steroid Diabetes

Dwight J. Ingle, Ph.D., Chicago*

The list of natural and synthetic compounds which are diabetogenic is already large and continues to grow apace. Steroids of the adrenal cortex are among the several hormones which are diabetogenic when given in excess. Evidence of this was first seen in patients having adrenal hypercorticism,[1] but the relationship between adrenal cortical functions and diabetes was not appreciated prior to the classical studies of Long and Lukens[2] and of Long, Katzin and Fry.[3] In these studies it was shown that diabetes is ameliorated by removal of the adrenal cortices and exacerbated by the administration of large amounts of the 11-oxygenated steroids. In related studies[4] it was shown that the diabetogenicity of cortisone and hydrocortisone is much greater than that of 11-desoxycorticosterone.

The production of severe hyperglycemia and glycosuria in the normal rat by the injection of cortisone was reported fifteen years ago.[5] In a further study[6] steroid diabetes was induced in normal rats by the injection of large amounts of either cortisone or hydrocortisone. A comparison was made between adrenal steroid diabetes and pancreatic diabetes in the rat. Among the features of adrenal steroid diabetes are the following: (1) a tendency toward insulin resistance which becomes remarkably severe in some individual animals; (2) a severe negative nitrogen balance; (3) absence of glycosuria during fasting; and (4) reversibility of the diabetes when the administration of hormone is stopped.

The question as to whether the adrenal cortices of the normal rat have the capacity to secrete diabetogenic amounts of steroids was easily answered. Ingle et al.[7,8] demonstrated steroid diabetes in normal rats given frequent injections of corticotropin. This hormone was found to be without a diabetogenic effect in adrenalectomized rats.[9]

Steroid diabetes has been demonstrated in other rodents.[10] The cat and dog are relatively resistant to steroid diabetes, although they are much more susceptible to the diabetogenicity of growth hormone than is the rodent. In 1949 Sprague et al.[11] described steroid diabetes in a 14-year-old boy with Cushing's syndrome. The diabetes was of unusual severity and was insensitive to insulin. Hydrocortisone was excreted in the urine in considerable quantities. Hyperplastic adrenal cortices were found at autopsy. In addition to its occurrence in some patients with Cushing's syndrome, steroid diabetes is known to develop in some patients who are given large amounts of either corticotropin or hydrocortisone and chemically related compounds.[12–14] Actually when these hormonal therapeutic agents are used judiciously, the incidence of steroid diabetes among patients is very small. Impairment in glucose tolerance can be demonstrated in some patients receiving large doses of these hormones. Changes in insulin tolerance are even less frequent.[15]

RECENT STUDIES

Conditions required for the development of steroid diabetes in the rat. The smallest dose of steroid which we have found to induce temporary glycosuria in any individual rat is 3 mg. of cortisone per day and 2 mg. of hydrocortisone per day. A daily dose of 10 mg. per rat causes at least a temporary glycosuria in almost all of the animals tested. The rat is more likely to develop steroid diabetes when it is force fed, than when it eats an equivalent amount of food ad libitum. The twice-daily force-feeding of a fluid diet represents a greater dietary load per unit time than does ad libitum eating. However, normal animals can tolerate at least twice the normal caloric requirement of a high carbohydrate fluid diet without the development of alimentary glycosuria.[16]

In our initial studies on steroid diabetes the rats were maintained on a high carbohydrate diet. Subsequent studies demonstrated that rats can be made to excrete glucose on any diet representing a normal caloric intake. Figures 1 and 2 illustrate the development of glycosuria in normal rats maintained on a high fat, low carbohydrate diet and given large doses of corticotropin[17] or of cortisone acetate.[18] Steroid diabetes cannot be induced in the fasting rat by the daily injection of up to 50 mg. of either cortisone or hydrocortisone per rat per day.

Duration of steroid diabetes in the rat. There is a latent period of at least three days between the beginning

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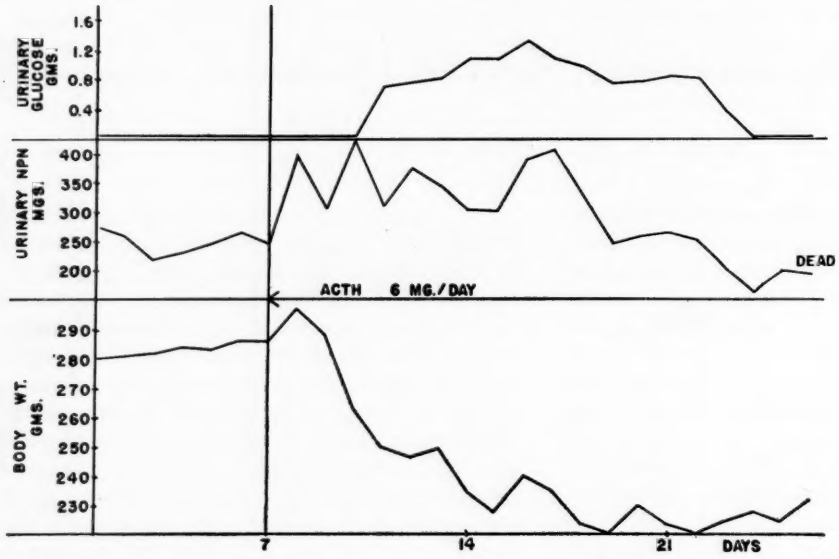

FIG. 1. Continuous injection of ACTH (Li) to a normal male rat force-fed a high fat diet.[17]

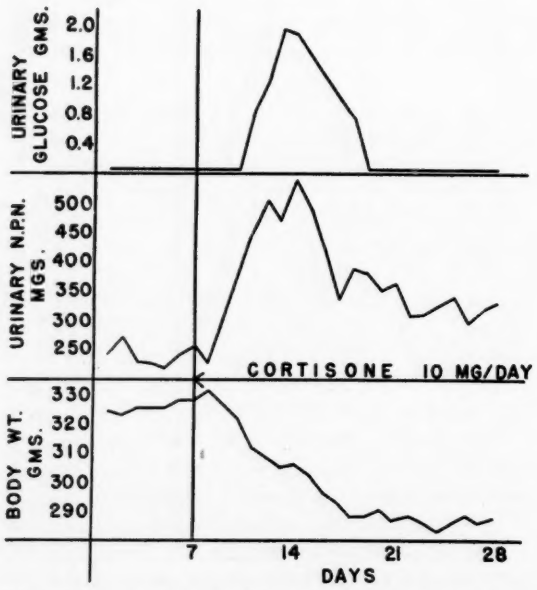

FIG. 2. Normal male rat force-fed a high fat, low carbohydrate diet.[18]

of administration of the steroid and the appearance of glycosuria. In earlier days I came to the possibly incorrect conclusion that steroid diabetes cannot be sustained in the normal rat. It is true that in most rats the diabetes may wax and wane, but with doses of 10 mg. of cortisone acetate per rat per day we have not seen the glycosuria disappear during periods up to six weeks. We have not studied such animals for longer periods due to the development of generalized infections in rats with severe hypercorticism. This complication could probably be prevented by treatment with antibiotics. In the rat the diabetes always disappears when the administration of the steroid is stopped.

Steroid diabetes in rats given cortisone and diethylstilbestrol. Estrogens are diabetogenic in the force fed rat. Cortisone and diethylstilbestrol were administered separately and in combination for fourteen days to normal rats which were force fed a medium carbohydrate diet. Doses of these drugs that failed to cause glycosuria when given separately did cause glycosuria when given simultaneously.[19] These results are illustrated in figure 3.

In similar experiments Engel et al. have shown additive diabetogenic effects of growth hormone and either corticotropin[30] or cortisone acetate.[31]

Level of liver glycogen in rats having steroid diabetes. The results of studies on levels of liver glycogen in animals having either alloxan or pancreatic diabetes have been highly variable. We find a very high level of liver glycogen in rats having steroid diabetes.[25] Male rats force fed a medium carbohydrate diet were injected with cortisone for seven days and the liver excised at the end of a 24-hour fast. The results are shown in figure 4.

Normalizing effects of adrenal steroids. Although the fasting "adrenally insufficient" animal is prone to develop hypoglycemia, the tolerance of such animals for carbohydrate can be shown to be less than normal if the

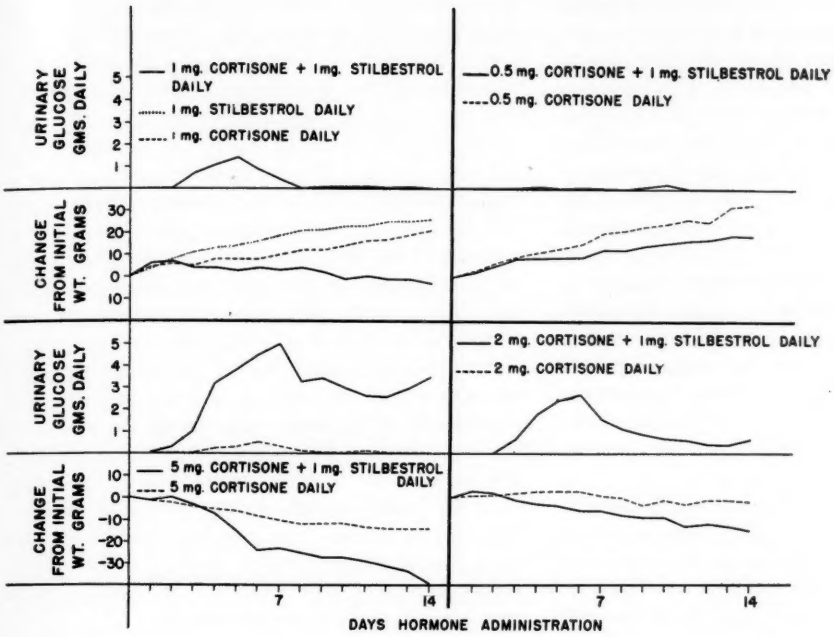
URINARY GLUCOSE GMS. DAILY
1 mg. CORTISONE + 1mg. STILBESTROL DAILY
1 mg. STILBESTROL DAILY
1 mg. CORTISONE DAILY
0.5 mg. CORTISONE + 1 mg. STILBESTROL DAILY
0.5 mg. CORTISONE DAILY
CHANGE FROM INITIAL WT. GRAMS
2 mg. CORTISONE + 1mg. STILBESTROL DAILY
2 mg. CORTISONE DAILY
5 mg. CORTISONE + 1 mg. STILBESTROL DAILY
5 mg. CORTISONE DAILY
DAYS HORMONE ADMINISTRATION

FIG. 3. Effects of diethylstilbestrol and of cortisone acetate given separately and in combination upon the glycosuria of normal force-fed rats. Averages, 6 to 12 rats per group.[19]

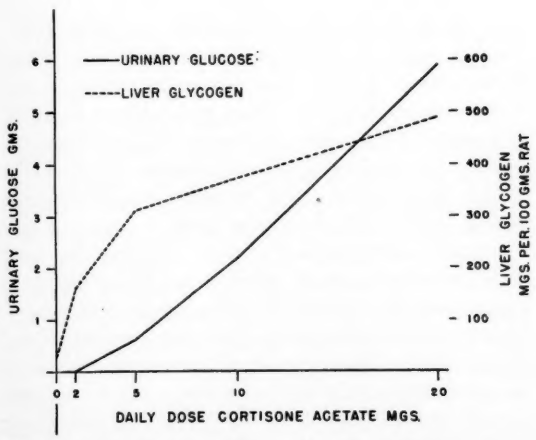

FIG. 4. Levels of urinary glucose and of liver glycogen as related to the dose of cortisone acetate. Averages, 6 to 16 rats per group.[25]

ability of the animal to assimilate an abnormally high load of carbohydrate is fully tested.[22, 23] In such cases treatment with adrenal cortical extract or steroid in physiological or normalizing amounts will improve the ability of the animal to adapt to carbohydrate excess. Only states of hypercorticism cause steroid diabetes. De Bodo and associates[24] have shown that the diabetes caused by the administration of growth hormone to hypophysectomized dogs is ameliorated by treatment with normalizing doses of adrenal steroids.

Suppression of experimental diabetes with adrenal steroids. The prolonged administration of small doses of 11-oxygenated steroids to partially depancreatized rats caused a marked decrease in the number showing glycosuria at the end of six months.[26] This protective action is associated with marked hypertrophy and hyperplasia of the pancreatic islets.

MECHANISMS OF STEROID DIABETES

Gluconeogenesis. The well-known effect of the 11-oxygenated adrenal steroids in stimulating gluconeogenesis from noncarbohydrate sources is an important factor in causing steroid diabetes. Some investigators believe that the accelerated formation of carbohydrate is solely responsible for steroid diabetes. I do not believe that an increase in gluconeogenesis can account for all of the glucose wasted during the diabetic state. Welt et al.[27] gave continuous intravenous infusions of solutions of C^{14} glucose into anesthetized fasted rats until constant levels of specific activity of urinary glucose were obtained in normal and cortisone-treated rats. From the specific activities of injected and excreted glucose and the known rate of injection, the rate of gluconeogenesis from sources not derived from the infused glucose was calculated. Rats having steroid diabetes induced by cortisone showed an approximately sevenfold increase over normal in the rate of gluconeogenesis. The rate of oxidation of glu-

cose to carbon dioxide did not appear to be significantly affected by treatment with cortisone. However, just prior to the experiment the magnitude of the glycosuria of the active fed animals represented an average of 262 mg. of glucose per rat per hour which is too great to be accounted for on the basis of the calculated rate of glucose production of 113 to 133 mg. per rat per hour. Values for glucose excretion up to 540 mg. per rat per hour have been observed[6] in steroid diabetic animals fed a high carbohydrate diet. On the basis of simultaneous nitrogen excretion data this degree of glycosuria was too great to be attributed solely to gluconeogenesis from protein. When these data are considered together with the finding that normal active rats can be adapted to utilize as much as 1,000 mg. of glucose per hour, it seems plausible that in the fed active rats having steroid diabetes there is contribution to the glycosuria from impairment in utilization of carbohydrate.

The evidence[27] that the rate of conversion of protein to carbohydrate is greater in steroid diabetes than in alloxan diabetes should be critically re-examined. Our rats having diabetes caused by cortisone were studied after the diabetes had existed for only a few days. They were still well nourished, and nitrogen loss had just reached its peak. The rats treated with alloxan had been diabetic for several weeks and were somewhat protein depleted although still vigorous. It is known that rats with steroid diabetes tend to re-establish nitrogen balance, or at least the rate of nitrogen loss declines. We have unpublished data to show that when the alloxan diabetic rat is treated with insulin until its proteins are repleted there is much greater gluconeogenesis from protein when insulin is withdrawn.

Inhibition of carbohydrate utilization. In addition to the failure of the known changes in gluconeogenesis to account for all of the glucose wasted into the urine in steroid diabetes, there are several lines of evidence that the 11-oxygenated steroids inhibit some phase of carbohydrate utilization. The studies of Price et al.[28] have shown that adrenal cortical extract can modify the hexokinase reaction by intensifying the inhibitory effect of anterior pituitary extract upon the action of insulin. An action at this level would not explain all of the effects of adrenal steroids upon carbohydrate metabolism, for these hormones act in the absence of the pituitary hormones and in the absence of insulin. There are other studies contributed to and reviewed by Bacila and Barron[29] showing that the metabolism of isolated tissues, especially anaerobic glycolysis, is modified by the adrenal steroids.

A decrease in respiratory quotient following the administration of adrenal steroids to patients with Addison's disease[30] may be due to suppression of carbohydrate utilization, but such effects have been infrequently reported. Boutwell and Chang[31] studied the utilization of radioactive glucose in normal and cortisone-treated mice. The amount of glucose utilized by the mice within four to six hours following the injection of cortisone was 65 per cent of normal. The reduction of glucose utilization was attributed to a depression in glucose oxidation as well as to the quantity of glucose lost to other metabolic pools.

Frawley[32] has noted an elevation of pyruvic acid without accompanying changes in glucose tolerance in patients with Cushing's syndrome and in patients receiving prolonged corticotropin or 11-oxygenated therapy.

Since the liver is the prinicipal site of gluconeogenesis we have studied the effect of cortisone, hydrocortisone, and adrenal cortical extract upon the glucose tolerance of the eviscerate rat.[33] Adrenal cortical extract caused a significant elevation of blood glucose above the average value for control animals during a period of 24 hours, but the two steroids were relatively ineffective. When large doses of either cortisone or hydrocortisone are given to the adrenalectomized-eviscerate rat without insulin there is suppression of glucose tolerance.

COMMENT

Steroid diabetes apparently involves both overproduction and under-utilization of carbohydrate. How are these effects related? Is one secondary to the other, or are both effects secondary to a more basic action? There is no apparent reason why acceleration of gluconeogenesis would cause suppression of carbohydrate utilization, although this order of causal relationship cannot be excluded. We[6, 35] have tested the possibility that control of steroid-induced glycosuria by insulin will suppress the accompanying rise in urinary nonprotein nitrogen. The reduction of glycosuria during insulin therapy did not suppress the nitrogen loss. Although very large doses of steroid cause insulin resistance, something more than inhibition of insulin action is involved since the diabetogenicity of these steroids is manifest in the absence of insulin.

Since the secretory activity of the adrenal cortices is increased during severe stress it has been imagined that any severe nonspecific stress should cause exacerbation of a diabetic state. Insofar as this problem has been studied there is little supporting evidence. Some stressors such as aspirin,[36] cold,[37] exercise,[38] and injections of dilute formaldehyde[39] cause a decrease in the glycosuria of diabetic rats. Infections may cause insulin resistance in diabetic patients,[40] and ethylenediamine causes exacerbation of the diabetes of partially depancreatized rats.[41] Some of our experiments[42-44] can be interpreted as evidence

that activation of the adrenal cortices during stress serves to meet an increased need for cortical steroids and does not cause hypercorticism.

The question as to whether the steroids of the adrenal cortex play an active etiologic role in diabetes mellitus has not been answered to the satisfaction of all clinical and laboratory investigators in the field. It is my opinion that the question will probably be answered in the negative for the following reasons: (1) There is little evidence of hypercorticism among patients with diabetes mellitus; (2) steroid diabetes is rare among the large population of patients now treated with larger than physiological doses of adrenal steroids or corticotropin; (3) steroid diabetes does not simulate diabetes mellitus in several important respects; and (4) steroid diabetes is reversible, i.e., it disappears when treatment with exogenous steroid or corticotropin is withdrawn.

SUMMARIO IN INTERLINGUA

Diabete Steroide Experimental

Diabete steroide involve apparentemente (1) hyperproduction e (2) hypoutilisation de hydratos de carbon. Que es le relation de iste effectos? Es le un secundari al altere, o es ambes secundari a un action plus fundamental? Nos vide nulle ration proque le acceleration del gluconeogenese causarea un suppression del utilisation de hydratos de carbon, sed il non es possibile excluder categoricamente un tal nexo causal. Nos ha studiate le possibilitate[6, 35] que le controlo insulinic de glycosuria a induction steroide supprime le accompaniante augmento del nonproteinic nitrogeno urinari. Le reduction del glycosuria durante le therapia a insulina non supprimeva le perdita de nitrogeno. Ben que grandissime doses steroide causa un resistentia a insulina, un factor in ultra del inhibition del action de insulina es involvite in iste situation, proque le diabetogenicitate de iste steroides es manifeste in le absentia de insulina.

Proque le activitate secretori del cortices adrenal es augmentate sub conditiones de sever stress, on ha speculate que omne nonspecific stress deberea causar un exacerbation del stato diabetic. In tanto que iste problema ha essite studiate, il non existe multe datos a supportar ille speculation. Certe stressantes—per exemplo aspirina,[36] rheuma,[37] exercitio,[38] e injectiones de dilutiones de formaldehydo—causa un reduction del glycosuria de rattos diabetic. Infectiones pote causar resistentia a insulina in patientes diabetic,[40] e ethylenediamina causa un exacerbation del diabete de partialmente pancreatectomisate rattos.[41] Certes inter nostre experimentos[42–44] pote esser interpretate como prova que le activation del cortices adrenal sub conditiones de stress servi a satisfacer le augmentate requirimentos de steroides cortical e non produce hypercorticismo.

Le question de saper si le steroides del cortice adrenal ha un active rolo etiologic in diabete mellite ha non ancora recipite un responsa que satisface omne le investigatores clinic e laboratorial in le campo. In mi opinion il es probabile que le responsa va esser negative. Mi rationes es le sequentes: (1) Il non ha multe datos indicante le presentia de hypercorticismo in patientes con diabete mellite. (2) Diabete steroide es rar inter le numerose patientes qui es hodie tractate con doses plus que physiologic de steroides adrenal o de corticotropina. (3) Diabete steroide non simula diabete mellite in certe aspectos importante. E (4) diabete steroide es reversibile, i.e., illo dispare quando le tractamento con steroide exogene o con corticotropina es supprimite.

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DISCUSSION

JOSEPH W. JAILER, M.D., (*New York*): Dr. Ingle will be the first to admit that we must not blindly fall into the trap of transferring data obtained in one species to another. I should like to discuss superficially, at least, what is known of the role of adrenal steroids in diabetes mellitus in man. The steroids which have been commonly used, of course, have been cortisone, hydrocortisone, and prednisone.

We do know, for example, that diabetes exists in hyperadrenalism and that 85 per cent of patients with Cushing's syndrome have at least decreased glucose tolerance. However, only about 25 per cent of patients with Cushing's syndrome have overt diabetes. When these patients are treated, the glucose tolerance may revert to normal in some cases but not in others, and those patients with overt diabetes, who may need some steroid replacement therapy, still may require insulin. Of course, the insulin requirement is greatly decreased. Consequently, one may readily conclude that the steroids play an important role in the diabetes of Cushing's syndrome.

Another interesting phenomenon in which the steroids may play a part in changing the course of diabetes is in pregnancy. As you know, many patients with diabetes show an exacerbation of their disease during pregnancy. Others have been known to develop diabetes during the course of pregnancy. There is circumstantial evidence that these manifestations may be due to increased corticoid secretion from the adrenals and perhaps even from the placenta. Increased urinary and plasma corticoids have been found during pregnancy, as determined by various methods. The adrenals also show an exaggerated response to ACTH during pregnancy, as determined by the response of plasma corticoids. This is in contrast to the normal. In addition, we have studied two pregnant Addisonian patients and have found a rise in plasma corticoids during pregnancy. That this rise is not due to the adrenals has been shown by the administration of ACTH, which causes no further rise in the plasma corticoids.

There is one more point I should like to make, and that concerns the role of adrenal steroids in diabetic acidosis or in coma. MacArthur and her collaborators have found excessive amounts of corticoids in the urine of patients in diabetic coma or severe acidosis, and we have had the experience of finding elevated plasma corticoid levels in just such patients. This might possibly explain the phenomenon of insulin resistance in these patients at that time. That this is in all probability the effect and not the cause is shown by the following data. When the blood sugar is brought down to normal and the acidosis corrected, the plasma corticoids are normal. In addition, MacArthur had withheld insulin from such patients and has shown that the development of acidosis precedes the increase in urinary corticoids. Consequently, one must conclude that acidosis is probably a stress which activates the pituitary-adrenal system and results in increased adrenocortical secretion.

The Influence of Heredity

The interest in hereditary bulimia which began with Danforth's work on yellow mice in 1927 has greatly increased with new work on other mammals.[1]

While studies of heredity in human obesity cannot be pursued under as rigorous conditions as in laboratory animals, there are a number of careful projects which reinforce the thesis that the constitution or the physical characteristics (shape and size) of the body are inherited but the amount of adipose tissue is not. The more convincing of these human studies are on identical (monozygotic) twins, because it is believed that such pairs are identical for all body traits. If there is a body-weight variation, it is presumably due to environmental, including psychological, factors. It was, in fact, found by von Verschuer in 1927 and Newman et al. in 1937 that the weight of identical twins varied more significantly than any other anthropologic measurements.[2, 3]

The differences in weight tended to be two to three times as great as the other trait variations. This was especially true for identical twins living apart under dissimilar conditions.

So again we find that the evidence from human genetics compels us to examine in closer detail the nature of environmental factors influencing appetite and habits of eating.

From the book *Modern Nutrition in Health and Disease* edited by Michael G. Wohl, M.D., and Robert S. Goodhart, M.D. Philadelphia, Lea & Febiger, 1955, Chapter "The Psychology of Appetite" by Henry W. Brosin, M.D., p. 77.

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Experimental Pituitary Diabetes

R. C. de Bodo, M.D., and N. Altszuler, Ph.D.,† New York*

It is well established from the studies of Houssay,[1] Young,[2] and others[3] that anterior pituitary extracts can produce diabetes. Although the diabetogenic effect of such extracts may have been due, in part, to their ACTH content, the production of diabetes by these extracts in the absence of the adrenal glands indicates that factors other than ACTH were also responsible for this effect.

An investigation was undertaken to determine which of the anterior pituitary hormones have an action on carbohydrate metabolism antagonistic to that of insulin and may be diabetogenic under pathological and certain experimental conditions. A hormone was considered to be anti-insulin if it ameliorated or abolished the insulin hypersensitivity of the hypophysectomized dog and if it changed its characteristic glucose tolerance toward a normal or diabetic type.

Of the anterior pituitary hormones studied, the three which have satisfied the above criteria are: ACTH, prolactin and growth hormone.

ACTH

The effect of ACTH treatment on the insulin hypersensitivity and glucose tolerance of hypophysectomized dogs was investigated.[4-6] Trained animals were used without anesthesia and in the postabsorptive state (17-18 hrs. fasted). Prior to the ACTH treatment all the animals exhibited the metabolic abnormalities characteristic of hypophysectomized animals. The ACTH* was administered four times daily at total daily dosages of 3-20 IU/kg. for a period of 23-125 days. The weights of the adrenal glands of most of these animals maintained on the large doses and prolonged treatment were within the normal range. The adrenal glands of all the treated animals showed evidence of high lipoid contents and varying degrees of hyperplasia.

The effect of an ACTH regimen on the insulin hypersensitivity of the hypophysectomized dog is presented in figure 1. It can be seen that upon continued administration of ACTH, the postabsorptive blood sugar was elevated and the exaggerated sensitivity to insulin was abolished, resulting in a normal response. Prolonged administration of ACTH produced a resistance to the hypoglycemic effect of insulin inasmuch as the response was less than that of the normal dogs. Concomitantly with these effects, the ACTH treatment changed the glucose tolerance of the hypophysectomized dogs, as shown in figure 2. In the untreated state, the operated animals manifested a low postabsorptive blood sugar, and following an intravenous glucose infusion, the blood sugar, after the initial rise, fell below the preinfusion level, thus resulting in a secondary hypoglycemia. The ACTH treatment elevated the low postabsorptive blood sugar, raised the peak sugar value and greatly diminished

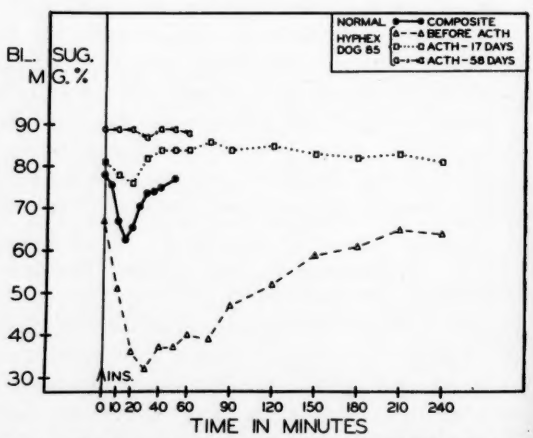

FIG. 1. Blood sugar curves produced by insulin (0.025 u/kg. i.v.) in normal dogs and in a hypophysectomized (Hyphex) dog before and during ACTH regimen. Insulin responses were tested after 17 and 58 days of ACTH regimen; Squibb ACTH C15Pr, 3 IU/kg./day, i.m. From Recent Progress in Hormone Research 8:533, 1953. New York, Academic Press Inc.

*ACTH and prolactin prepared and kindly supplied by Dr. R. W. Bates, formerly of Squibb and Sons.

Presented at the Symposium on The Pituitary-Adrenal System and Diabetes Mellitus, presented by the New York Diabetes Association, Oct. 27, 1955.

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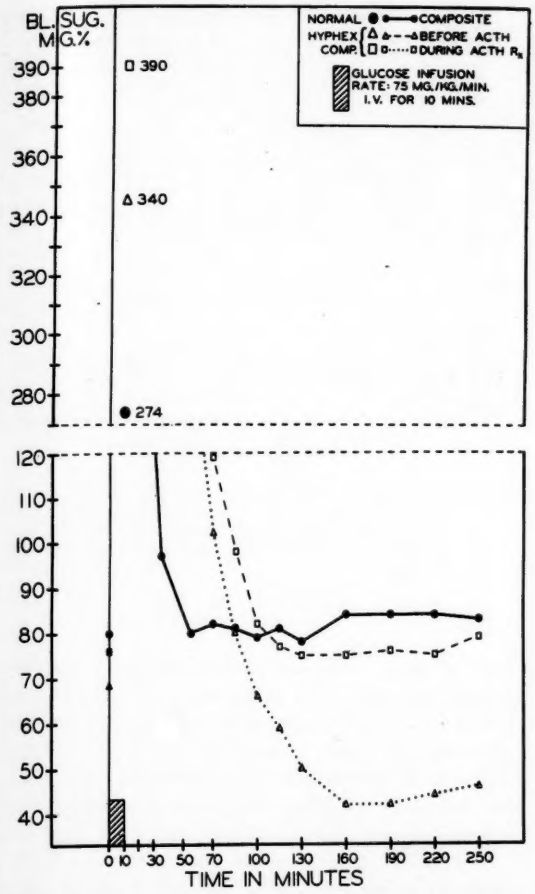

FIG. 2. Composite blood sugar curves produced by intravenous glucose (0.75 g./kg.) in normal dogs and in hypophysectomized (Hyphex) dogs before and during ACTH regimen; Squibb ACTH, 3-20 IU/kg./day, i.m., for a period of 23-125 days.

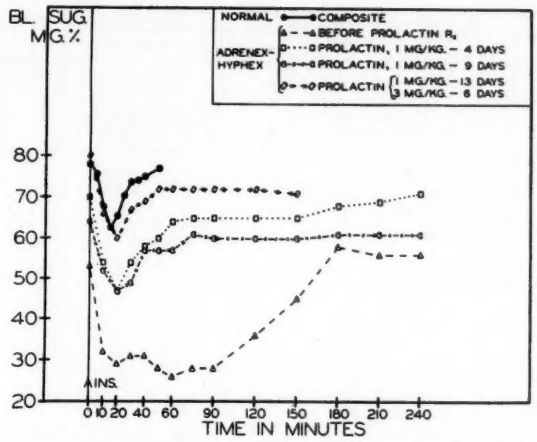

FIG. 3. Blood sugar curves produced by insulin (0.025 u/kg. i.v.) in normal dogs and in an adrenalectomized-hypophysectomized (Adrenex-Hyphex) dog before and during a prolactin regimen. Insulin responses were tested after 4, 9 and 19 days of prolactin regimen; Squibb prolactin Nr. 71713 (1 mg.=20-25 IU) given in doses of 20-75 IU/kg./day, i.m.

or abolished the secondary hypoglycemia. Thus it can be seen that ACTH abolished the insulin hypersensitivity of the hypophysectomized dogs and changed their glucose tolerance toward normal and, therefore, can be regarded as a physiological insulin antagonist.

PROLACTIN

The second anterior pituitary hormone shown to exert an anti-insulin effect is prolactin. Initially this effect was demonstrated in hypophysectomized dogs.[7] However, since the available prolactin preparations were not completely free of ACTH, hypophysectomized-adrenalectomized dogs were used to exclude any possible action of the ACTH contaminant.[7] The prolactin was administered in dosages of 20-125 IU/kg., once daily, for a period of 19-30 days. The effects of prolactin treatment on the insulin hypersensitivity of hypophysectomized-adrenalectomized dogs are shown in figure 3. As a result of the treatment, the insulin hypersensitivity of these animals was diminished, and at the higher dosage levels the response to insulin was restored practically to normal. Similarly, prolactin administration diminished the secondary hypoglycemia observed in the glucose tolerance tests of these animals, as shown in figure 4. Thus prolactin can abolish the exaggerated insulin response of the hypophysectomized-adrenalectomized (and hypophysectomized) dogs and partially restore their glucose tolerance toward normal and, therefore, may be of physiological significance as an insulin antagonist.

GROWTH HORMONE

A third anterior pituitary hormone having an effect antagonistic to that of insulin is growth hormone (or possibly a hormone closely linked with it, but as yet not separated from it). The administration of growth hormone in dosages of 1.0 mg./kg./day abolished the insulin hypersensitivity of hypophysectomized dogs in 3-7 days.[8, 6, 9, 10] Smaller doses can also exert this effect, as shown in figure 5. The dose in these experiments was 0.3 mg./kg./day, but even 0.02 mg./kg./day exerted some anti-insulin effects,[6, 9] thus attesting to the great sensitivity of the hypophysectomized animal to growth hormone. When the growth hormone abolished the insulin hypersensitivity of hypophysectomized dogs, it concomitantly changed their glucose tolerance to a diabetic

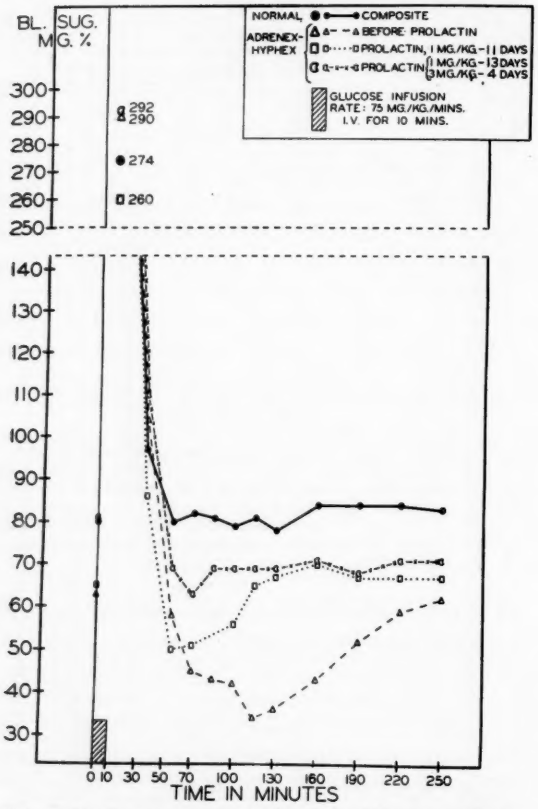

FIG. 4. Blood sugar curves produced by intravenous glucose (0.75 g./kg.) in normal dogs and in an adrenalectomized-hypophysectomized (Adrenex-Hyphex) dog before and during a prolactin regimen. Glucose tolerance tests performed after 11 and 17 days of prolactin regimen; Squibb prolactin Nr. 71713 (1 mg.=20-25 IU), 20-75 IU/kg./day i.m.

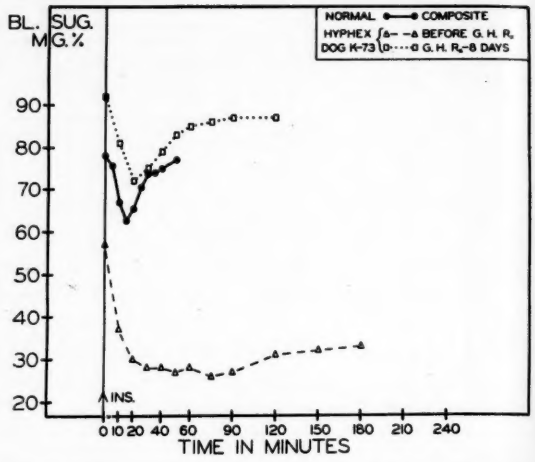

FIG. 5. Blood sugar curves produced by insulin (0.025 u/kg., i.v.) in normal dogs and in a hypophysectomized (Hyphex) dog before and during growth hormone regimen; Armour, lot #22KRI, 0.3 mg./kg./day, i.m., given for 8 days.

type.[8, 6, 9, 10, 11] The growth hormone diabetes in the hypophysectomized dog was characterized by the following: (a) the postabsorptive blood sugar was elevated (but never above 125 mg. per cent level); (b) the glucose tolerance was diminished, as evidenced by a considerably retarded fall of blood glucose from the peak level at the end of an intravenous glucose infusion; however, (c) glycosuria or acetonuria was not present. As shown in figure 6, the administration of growth hormone in dosages of 1 mg./kg./day resulted in a diabetic type of glucose tolerance after only three days. Only rarely was it possible during a growth hormone regimen to observe a normal glucose tolerance[11] which, if it did occur, lasted only for a few days before it became diabetic.

In addition to the diabetes, the growth hormone treatment resulted in toxic manifestations, e.g., anorexia, refusal of food, vomiting of force-fed food, weakness, lethargy and death. The above effects were observed following the administration of growth hormone preparations obtained from various sources: Armour Laboratories, E. R. Squibb and Sons, Connaught Laboratories, and Dr. C. H. Li. When the growth hormone dose was reduced, thereby making possible prolonged administration without the toxic effects, the animals developed a resistance to the hormone as reflected by its diminished anti-insulin effect.[11] Thus it was not feasible to establish a replacement therapy with growth hormone in the hypophysectomized dog.

GROWTH HORMONE IN CORTISONE OR HYDROCORTISONE TREATED HYPOPHYSECTOMIZED DOGS

The administration of growth hormone without inducing diabetes and toxicity was possible, however, when cortisone or hydrocortisone was administered prior to and concomitantly with the growth hormone. As can be seen from figures 7 and 8, the steroids per se elevated the postabsorptive blood sugar of the hypophysectomized dogs, diminished the secondary hypoglycemia of the glucose tolerance tests, and, of significant importance, accelerated the disposal of the glucose load (if it was retarded in the untreated state), as indicated by the rapid fall of the blood sugar from the peak value at the end of the glucose infusion. In addition, the steroids ameliorated the insulin hypersensitivity of the hypophysectomized dogs (figure 9).

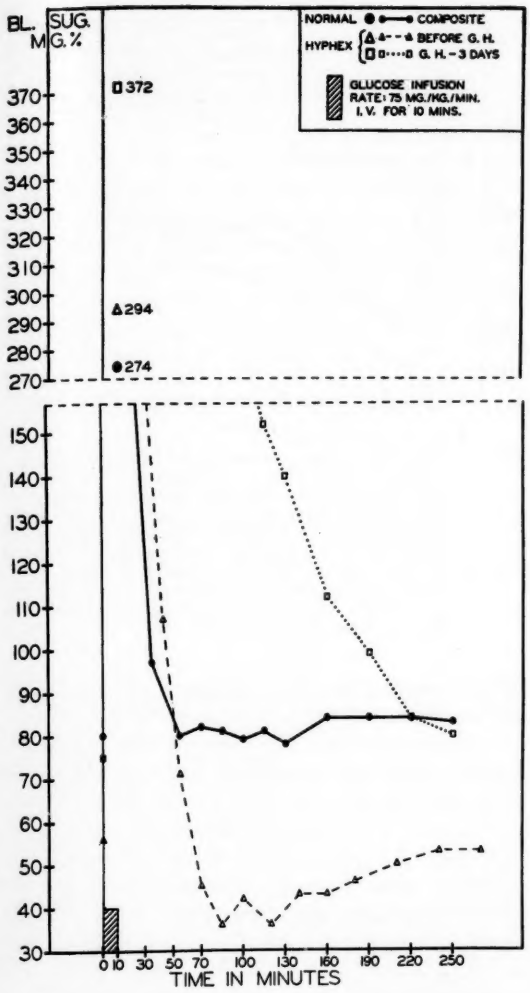

FIG. 6. Blood sugar curves produced by intravenous glucose (0.75 g./kg.) in normal dogs and in a hypophysectomized (Hyphex) dog during the untreated state and after 3 doses of growth hormone; Armour, lot #22KR1, 1 mg./kg./day, i.m. From DIABETES 3:90, 1954.

When the steroid regimen was changed to a combined growth hormone-steroid regimen, the growth hormone antagonized the insulin effect still further (figure 9), elevated the postabsorptive blood sugar to higher levels and abolished the secondary hypoglycemia (figures 7 and 8), but significantly, without producing diabetes or toxicity. Since the growth hormone was administered in a dose that was diabetogenic when given alone to hypophysectomized dogs, it is clear that the adrenal steroids exerted a beneficial effect in the combined therapy.

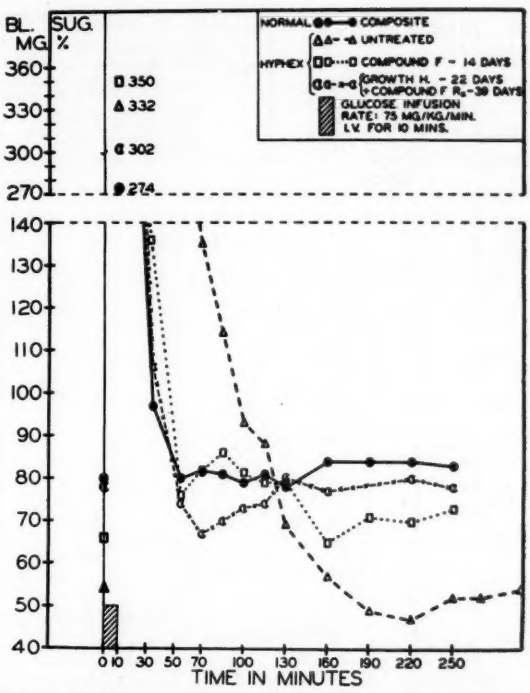

FIG. 7. Blood sugar curves produced by intravenous glucose (0.75 g./kg.) in normal dogs and in a hypophysectomized (Hyphex) dog, (a) during the untreated state, (b) during hydrocortisone regimen, and (c) during a combined hydrocortisone - growth hormone regimen. Hydrocortisone (0.83 mg./kg./day, i.m.) given for a total of 39 days. Growth hormone regimen (Armour, lot #J21609R, 1.0-1.5 mg./kg./day, i.m.) was begun on the 18th day of hydrocortisone regimen and continued concomitantly for 22 days. From The Hypophyseal Growth Hormone, Nature and Actions, by Smith et al. Copyright, 1955. Blakiston Div., McGraw-Hill Book Co.

EFFECTS OF CORTISONE OR HYDROCORTISONE ON CARBOHYDRATE METABOLISM OF HYPOPHYSECTOMIZED DOGS AS STUDIED WITH C^{14} GLUCOSE

From recent studies, carried out in collaboration with Drs. R. Steele and J. S. Wall, considerable additional information was gained concerning the effect of steroid therapy in hypophysectomized dogs. In these experiments, minute amounts of uniformly labeled, highly radioactive glucose were administered by an initial intravenous priming injection and a continuous intravenous infusion lasting 4-5 hours. The amount of glucose administered was insignificant (not more than 3 mg. during the whole experiment) and did not raise the blood glucose level. On the other hand, the specific activity of the priming glucose injection was high and produced rapidly a readily measurable level of radioactivity in the blood glucose. The constant infusion of appropriately smaller

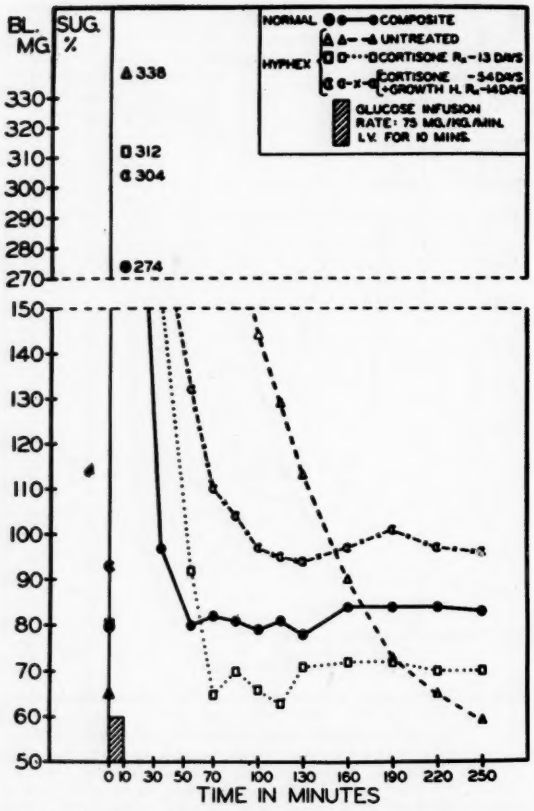

FIG. 8. Blood sugar curves produced by intravenous glucose (0.75 g./kg.) in normal dogs and in a hypophysectomized (Hyphex) dog, (a) during the untreated state, (b) during cortisone regimen, and (c) during a combined cortisone-growth hormone regimen. Cortisone (1 mg./kg./day, i.m.) given for a total of 54 days. Growth hormone regimen (Armour, lot #J21609R, 1 mg./kg./day, i.m.) was begun on the 40th day of cortisone regimen and continued concomitantly for 14 days. From The Hypophyseal Growth Hormone, Nature and Actions, by Smith et al. Copyright, 1955. Blakiston Div., McGraw-Hill Book Co.

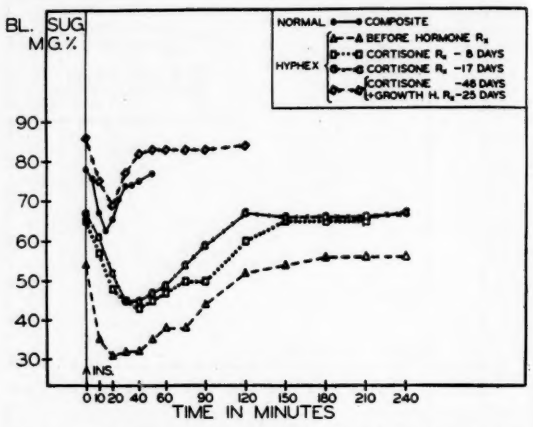

FIG. 9. Blood sugar curves produced by insulin (0.025 u/kg., i.v.) in normal dogs and in a hypophysectomized (Hyphex) dog, (a) during the untreated state, (b) during cortisone regimen (after 8 and 17 days) and (c) during a combined cortisone-growth hormone regimen. Cortisone (1.0-1.4 mg./kg./day, i.m.) given for a total of 46 days. Growth hormone regimen (Squibb, #C_5I_{55}, 1.0-1.5 mg./kg./day, i.m.) was begun on the 22nd day of cortisone regimen and continued concomitantly for 25 days. From DIABETES 3:90, 1954.

amounts of C^{14} glucose served to label the endogenous glucose entering the plasma and thus maintain the radioactivity of the plasma glucose near the level set by the priming dose. Of the several parameters of carbohydrate metabolism which were determined, the pertinent ones for this presentation are the size of the glucose pool and its rate of replacement. The glucose pool may be defined as the amount of glucose which dilutes the administered C^{14} glucose, and the replacement rate of the body glucose as the rate of inflow of endogenous glucose from the liver into the plasma. Since in the course of these experiments the blood sugar concentration remained unchanged and no sugar was excreted by the kidneys, it must be assumed that the replacement rate equalled the rate of glucose uptake, i.e., utilization by the tissues.

It can be seen from table 1 that the untreated unanesthetized hypophysectomized dogs had a smaller glucose pool and a considerably reduced body glucose replacement rate as compared to the normals. It should be emphasized that in the untreated hypophysectomized dogs the rate of inflow of glucose from the liver was not only very low but it was not increased appreciably when the blood sugar level was drastically reduced. In experiments in which a small dose of insulin was administered to hypophysectomized dogs, the precipitous fall in blood sugar did not evoke a significantly increased inflow of glucose from the liver into the plasma, in contrast to the marked response observed in the normal animals.

The hypophysectomized dogs on cortisone or hydrocortisone regimen showed an increase in the glucose pool and its replacement rate (table 1). Since in the course of the five-hour experiments the increased inflow of glucose from the liver into the plasma occurred in the absence of blood sugar changes, it is evident that the hormone treatment also increased the glucose uptake by the tissues. This observed effect is in harmony with the findings cited above that the adrenocortical steroids accelerated the disposal of glucose in the glucose tolerance test of hypophysectomized dogs. In these steroid-treated animals, with their glucose turnover restored toward normal, the small dose of growth hormone was no longer

diabetogenic. Similarly, this dose of growth hormone was not diabetogenic in normal dogs.

SENSITIVITY TO GROWTH HORMONE

As can be seen from table 2, the hypophysectomized dog is much more sensitive to the diabetogenic effect of growth hormone than the normal one. The treatment with growth hormone in daily doses of 1.0 mg./kg. produced in the hypophysectomized dog diabetes in 3-7 days, but its development was always prevented by the C-11, 17-oxycorticosteroids, administered in doses which raised the low glucose turnover of these animals toward normal. In the normal dog, daily growth hormone doses of 1.0 mg./kg. did not alter its glucose tolerance, and the dose had to be raised to 3 mg./kg. before diabetes was observed. At this time the addition of cortisone or hydrocortisone to the regimen, in doses which in themselves did not alter the glucose tolerance, exacerbated the diabetes. This effect differs from that observed in the hypophysectomized dogs. Whether or not the steroid therapy will improve or exacerbate the growth hormone diabetes appears, therefore, to be dependent on the conditions under which it is administered.

TABLE 1

Glucose pool and its replacement rate in normal dogs and in hypophysectomized (Hyphex) dogs before and during cortisone regimens

Dog status	Number of experiments	Average venous plasma glucose mg.%	Glucose pool g./kg. b.w.	Glucose replacement rate g./m²/hr.
Normal	10*	107	0.284±0.011†	3.80±0.19
Hyphex Before cortisone Rx	3‡	90	0.163±0.015	2.12±0.34
Hyphex On cortisone Rx§	3‡	104	0.214±0.035	3.41±0.31

* on 7 animals
† standard error of mean
‡ on 3 animals
§ dose: 0.8-1.5 mg./kg./day for 8-16 days

TABLE 2

Influence of growth hormone and cortisone on the glucose tolerance of normal and hypophysectomized (Hyphex) dogs

Dog status	Hormone treatment: Growth hormone mg./kg./day	Hormone treatment: Cortisone mg./kg./day	Glucose tolerance
Normal	1.0	–	Normal
	3.0	–	Diabetic
	–	3.0	Normal
	3.0	3.0*	Severely diabetic
Hyphex	1.0	–	Diabetic
	–	0.8-1.5	Normal
	1.0	0.8-1.5*	Normal

* Administered for several days prior to, and then concomitantly with growth hormone.

DISCUSSION

Evidence has been presented that three of the anterior pituitary hormones: ACTH, prolactin and growth hormone, can abolish the insulin hypersensitivity of hypophysectomized dogs. Two of these, ACTH and prolactin, were shown to accomplish this without producing diabetes, whereas the anti-insulin effect of growth hormone was invariably accompanied by diabetes and toxicity. These untoward effects of growth hormone were prevented by the prior and simultaneous administration of cortisone or hydrocortisone. These beneficial effects of the steroids in the hypophysectomized animals are reflected in the increased glucose inflow from the liver and the increased glucose uptake by the tissues. The sensitivity of the hypophysectomized animals to the diabetogenic effects of growth hormone appears to be related to their low glucose turnover rather than the limited availability of the adrenocortical hormones. This suggestion is substantiated by the observations that adrenalectomized dogs, maintained on minimal doses of DOCA, exhibited a normal glucose turnover (perhaps due to some compensatory mechanism), and that their sensitivity to growth hormone was much less than that of the hypophysectomized animals.

In earlier publications it was postulated that if growth hormone is active in carbohydrate metabolism as a physiological blood sugar raising agent and insulin antagonist, it should, in proper dosages, ameliorate or abolish the insulin hypersensitivity of the hypophysectomized animal, without producing diabetes and toxicity.[6, 9, 10] The findings cited above have necessitated a modification of this supposition. Ideally, it should be possible to evaluate the physiological role of growth hormone in an animal deprived of its endogenous growth hormone but still possessing the remaining pituitary hormones. Since at present such a preparation is not available, in lieu of it, the steroid-treated hypophysectomized animal seems to be more suitable for this purpose than the untreated hypophysectomized animal. In these steroid-treated hypophysectomized animals, the added growth hormone still exerted an anti-insulin effect and raised the blood glucose level but without producing diabetes or toxicity. These observations lend further support to the contention that growth hormone is a physiological insulin antagonist.

The ability of the adrenal oxycorticosteroids to prevent or exacerbate the growth hormone diabetes warrants further comment. In the hypophysectomized dogs, as shown above, these steroids caused an increased inflow

of glucose from the liver into the plasma and consequently an increased glucose uptake by the tissues. It is reasonable to assume that this increased inflow of glucose stimulated an increased secretion of insulin which, in turn, antagonized the diabetogenic effect of growth hormone. Thus, under these conditions, the steroids exerted a beneficial effect. On the other hand, when the insulin secreting ability was greatly impaired or exhausted, e.g., in diabetes produced by alloxan or by large doses of growth hormone in intact animals, the administration of the oxycorticosteroids exacerbated the diabetes. Thus, whether the oxycorticosteroids will prevent or exacerbate the growth hormone diabetes appears to depend on the insulin secreting ability of the animal.

Since ACTH exerts its effects through the adrenal cortex, similar inferences may be made for ACTH as for the oxycorticosteroids. Furthermore, it may be assumed that the other two anterior pituitary insulin antagonists, prolactin and growth hormone, can be diabetogenic when present in excessive amounts and the insulin secreting ability of the animal is impaired.

SUMMARY

1. The insulin hypersensitivity of hypophysectomized dogs could be abolished by ACTH, prolactin or growth hormone. ACTH and prolactin accomplished this without producing diabetes, whereas growth hormone caused diabetes and toxicity concomitantly with its anti-insulin effect.

2. These untoward effects of growth hormone were prevented by prior and simultaneous administration of C-11, 17-oxycorticosteroids.

3. The hypophysectomized dog, in the postabsorptive state, was shown, by means of a tracer technic using C^{14} glucose, to have a smaller glucose pool, a lower rate of inflow of glucose from the liver into the plasma, and a lower rate of glucose uptake by the tissues than the normal dog.

4. The C-11, 17-oxycorticosteroid regimen increased the small glucose pool and low glucose replacement rate of the hypophysectomized dogs toward normal.

5. The hypophysectomized dogs were shown to be more sensitive to the diabetogenic effects of growth hormone than the normal dogs, and this sensitivity was abolished by the administration of the C-11, 17-oxycorticosteroids.

6. In normal dogs, doses of growth hormone much larger than those used in the hypophysectomized animals were necessary to produce diabetes, and the administration of the oxycorticosteroids at that time exacerbated the diabetes.

7. It is proposed that the C-11, 17-oxycorticosteroids administered to hypophysectomized dogs, by increasing the inflow of glucose from the liver into the plasma, stimulated the secretion of insulin which, in turn, antagonized the diabetogenic effects of growth hormone.

8. It is suggested that the insulin secreting ability of the animal determines whether the C-11, 17-oxycorticosteroids will prevent or exacerbate the growth hormone diabetes.

SUMMARIO IN INTERLINGUA

Diabete Pituitari Experimental

1. Esseva constatate que le hypersensibilitate a insulina in hypophysectomisate canes poteva esser abolite per ACTH, prolactina, o hormon de crescentia. ACTH e prolactina attingeva iste effecto sin producer diabete, durante que hormon de crescentia causava diabete e toxicitate in concomitantia con su effecto anti-insulinic.

2. Iste negative effectos de hormon de crescentia esseva prevenibile per le previe o simultanee administration de C-11,17-oxycorticosteroides.

3. Per medio de un technica traciatori que utilisava glucosa a C^{14}, il esseva possibile monstrar que hypophysectomisate canes in le stato postabsorptive es characterisate in comparation con canes normal per un reducite reservoir de glucosa, un reducite influxo de glucosa ab le hepate a in le plasma, e un reducite acceptation de glucosa per le histos.

4. Le administration de C-11,17-oxycorticosteroide augmentava le basse nivello del reservoir de glucosa e le basse grados del reimplaciamento de glucosa in hypophysectomisate canes usque a valores normal.

5. Le hypophysectomisate canes se monstrava plus sensibile al effectos diabetogene de hormon de crescentia que esseva le caso in canes normal, sed iste sensibilitate esseva abolite per le administration del C-11,17-oxycorticosteroides.

6. Le doses de hormon de crescentia necessari pro effectuar diabete esseva multo plus grande in canes normal que in canes hypophysectomisate. Le simultanee administration del oxycorticosteroides a canes normal exacerbava le diabete.

7. Nos opina que le administration de C-11,17-oxycorticosteroides a hypophysectomisate canes, per augmentar le influxo de glucosa ab le hepate a in le plasma, stimulava le secretion de insulina, lo que, de su parte, antagonisava le effectos diabetogene de hormon de crescentia.

8. Nos stipula le possibilitate que le capacitate del animal de secerner insulina determina si le C-11,17-oxycorticosteroides va prevenir o exacerbar le diabetogenicitate de hormon de crescentia.

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DISCUSSION

ABRAHAM WHITE, PH.D., (*New York*): It has been my privilege on several occasions to listen to Dr. de Bodo and the presentation this morning reaffirms my previous experiences, namely, that one is always impressed with the clarity of the presentation, the care of the experimentation, and the contribution which the data make to existing information.

It is always easier for a discussant to ask questions than to provide additional data, and some of these questions may seem to carry the impression that the interrogator is somewhat impatient that additional experiments have not been done. It is always simpler to suggest additional work for other people. I trust that my remarks will not be construed in that light but rather that they will indicate that I have indeed been stimulated by this presentation.

There appear to be three phases with respect to our understanding of diabetes, particularly from the experimental point of view, and Dr. de Bodo's investigations fall into the second phase; namely, that dealing with the pituitary. The first phase concerns the work on the pancreas, and the third phase, which we are now entering, concerns the work on the liver and the effects of glucagon and of insulinase and of insulinase inhibitor.

I propose to direct my comments to the three phases of the work which have been described; namely, ACTH, growth hormone, and prolactin, and perhaps I might just open with the semantic comment that inasmuch as what Dr. de Bodo has presented is concerned with the effects of ACTH, growth hormone, and prolactin on metabolic processes, rather than a direct effect of those hormones on insulin per se, perhaps one should delete the term "anti-insulin effect" and talk about "anti-insulin effects," because what we are discussing are actually the effects produced which may be contra to those of insulin rather than a direct action on the hormone.

The work with ACTH perhaps contains a paradox which I hope Dr. de Bodo will discuss; namely, that in the hypophysectomized animal ACTH—given, it is true, in large doses in order to restore the atrophied adrenal cortex initially and subsequently to exert its metabolic effects—is diabetogenic, and yet we have seen that in the hypophysectomized animal the effects of cortisone, if anything, are antidiabetogenic. If one makes the assumption that ACTH is concerned with the release of cortisone-like compounds from the adrenal cortex, one might come to the conclusion that perhaps this is a paradoxical situation.

Perhaps one might also ask what the effects of cortisone are on glucose disposal and replacement in a hypo-

physectomized animal which is not given growth hormone at the same time. Is this restoration of glucose disposal and replacement in a growth hormone-treated animal given small amounts of cortisone a permissive action of cortisone, as described by Dr. Ingle, or is this a physiological effect over and beyond that expected from the restoration to normal adrenal status of the animal that has been hypophysectomized?

One interesting finding that Dr. de Bodo revealed is the observation that one or more pituitary factors were intimately concerned with the regulation of the activity of the liver in terms of the over-all phenomena which he has discussed.

Turning to growth hormones, is there any physiological basis or explanation at the present time for the exceedingly toxic effects of growth hormone in the hypophysectomized animal? The greater degree of effectiveness of the growth hormone in producing diabetes in the hypophysectomized animal as compared to the normal animal raises the question of the contribution of the growth hormone to stimulating increased resistance to its diabetogenic effect. Does the pituitary exert an effect upon the pancreas so that in the normal animal the injection of growth hormone is counteracted by an accelerated production of insulin by the pancreas?

With respect to the work on prolactin and the diabetes produced by prolactin in the hypophysectomized adrenalectomized animal, one might ask, what is the mechanism of this action? There are conflicting reports with respect to the effect of prolactin upon the insulin content of the pancreas. Is the effect of prolactin mediated by the pancreas? Do we have histologic changes of the pancreas in the prolactin-treated animals? Or is the effect of prolactin, let us say, in pregnancy or in the female animal, mediated by way of a persistent corpus luteum, which could produce progesterone or progesterone-like compounds, which have been shown by Dr. Ingle to be diabetogenic?

Finally, where does the thyroid gland fit into this overall picture? Since the thyroid does influence the rate of transport of glucose into cells, or across membranes, as one can show, for example, in the gastrointestinal tract, what is the influence of the thyroid in determining these diabetogenic manifestations, particularly in an animal in which thyroid functioning has been depressed by prior hypophysectomy?

DR. DE BODO: As regards the effect of ACTH, in our experiments there is no paradox between the effects of ACTH and the adrenocortical hormones. They both restored the glucose tolerance of the hypophysectomized dog to normal. The experiments which I showed with ACTH did not reveal diabetes; however, diabetes can be produced with large doses of ACTH. It is very likely that the adrenal steroids, if given in sufficiently large doses, would also produce diabetes. In the present experiments only small amounts of the steroids were used.

Whether or not the adrenal steroids will prevent the "growth hormone diabetes" or exacerbate it appears to depend on the insulin secreting ability of the animal. In the hypophysectomized dog, where the glucose turnover is low, the continued administration of the steroids increased the inflow of glucose from the liver into the plasma, and thereby may have stimulated a greater insulin secretion, which would then antagonize the diabetogenic effect of growth hormone. By contrast, when these steroids were administered to animals whose insulin secreting ability was impaired, as in those made diabetic with alloxan or large doses of growth hormone, they made the diabetes worse.

As to whether the cortisone action observed in the hypophysectomized animals is permissive or not—this question can not be answered with certainty. In general one obtains a graded response to increasing doses of cortisone, as seen in the glucose tolerance and sensitivity tests in the hypophysectomized animal.

The mechanism of the toxicity associated with the administration of growth hormone in the hypophysectomized dog is not clear. Such animals do not exhibit a diabetic acidosis. It is possible that the administered growth hormone by inhibiting the glucose utilization and by enhancing protein synthesis and fat mobilization may so disturb the precarious metabolic existence of the hypophysectomized animal that it is unable to adjust to it. This may be the underlying cause of the toxic effects of growth hormone.

The mode of action of prolactin as an insulin antagonist is yet to be elucidated. We have no evidence as to whether prolactin acts directly or indirectly on the pancreas.

As regards the effects of the thyroid on the carbohydrate metabolism of hypophysectomized dogs, in our experience thyroxine, although administered in large doses to these animals, did not alter greatly their insulin hypersensitivity.

Endocrine Relationships in Carbohydrate Metabolism

In Vitro Observations

M. E. Krahl, Ph.D., Chicago*

Extensive observations have been made on carbohydrate utilization of four tissues under in vitro conditions: muscle, adipose tissue, liver, and mammary gland. The present paper deals briefly and pragmatically with the first three of these tissues, with emphasis on findings which may clarify events in the whole animal. A complete review will not be attempted, as the subject has been treated extensively elsewhere.[1-7] Experiments on mammary tissue have been reviewed by Folley.[8]

The relation of insulin and other hormones to glucose uptake and output in individual tissues will be discussed first. Then two other functions, fat synthesis and peptide synthesis, which are correlated with glucose uptake, will be taken up.

GLUCOSE UPTAKE AND OUTPUT BY TISSUES

The severely diabetic subject uses less glucose, and produces more glucose from noncarbohydrate precursors, than the normal. Stetten, Ingle, and co-workers[9, 10] found 11.5 mg. glucose per 100 gm. rat per hour to be oxidized to carbon dioxide by the normal, as compared to 4.5 mg. by the diabetic rat. Concurrently, 17.5 mg. per 100 cc. glucose were formed from endogenous sources by the normal, and 43.3 mg. per 100 cc. by the diabetic. The effects of insulin excess and deficiency on individual tissues may now be considered.†

Striated Muscle. Best[11] found glucose uptake of the cat hind limb to be increased from 0.38 to 0.67 mg. per gm. per hour by addition of insulin to the perfusion fluid. Gemmill[12] obtained an increase in glucose uptake by the excised rat diaphragm from 1.8 to 3.4 mg. per gm. per hour when insulin was added to the incubation medium in vitro. Of the glucose disappearing, about 60 per cent was recovered as glycogen. These experiments, proving that insulin increases glucose uptake by muscle, have now been confirmed and extended by numerous investigators.[1]

Muscle from diabetic animals, on the other hand, was observed to have a lower than normal glucose uptake;[13, 14] the lowered rate was increased toward normal by addition of insulin in vitro. Knowlton and Starling in 1912[15] found glucose uptake in a diabetic dog heart-lung preparation to be nearly zero as compared to the normal rate of 4 mg. per gm. of heart per hour; the normal rate was restored by adding an acid-alcohol extract of pancreas to the perfusion fluid. This finding was confirmed and extended by Cruickshank.[16] Similarly, glucose uptake by diabetic rat diaphragms was 0.8 mg. per gm. per hour as compared to the normal of 1.8. Addition of insulin in vitro raised the rate to 1.4, i.e. toward, but not to, normal.[13]

Glucose uptake of diaphragms from hypophysectomized rats was higher than normal during the period from 10 to 30 days after operation,[14, 17] average rates being 2.7 mg. per gm. per hour for the normal under these experimental conditions and 4.3 for the hypophysectomized. Hypophysectomy also allowed the glucose uptake of diabetic muscle to return toward normal.[14]

Glucose uptake of diaphragms from adrenalectomized rats was slightly above normal, but not as markedly as in the case of those from hypophysectomized rats.[13, 14] Adrenalectomy, like hypophysectomy, allowed glucose uptake by diabetic diaphragms to return toward normal.

The nature of the factors which regulate glucose uptake in muscle is now under study. The muscle from the hypophysectomized-adrenalectomized rat, suspended in a balanced salt solution,[13, 14] was still responsive to insulin added in vitro (figure 1); the maximal rate attainable with insulin was approximately the same for muscle from normal, from hypophysectomized, or from hypophysectomized-adrenalectomized rats. If this maximal rate with added insulin is considered to represent the maximal capacity of the muscle to use glucose, then there is experi-

†The absolute rates of glucose uptake vary with the experimental conditions employed. Comparisons made with tissues from hormonally unbalanced animals are valid only with respect to the concurrent controls.

Presented at the Symposium on The Pituitary-Adrenal System and Diabetes Mellitus, presented by the New York Diabetes Association, Oct. 27, 1955.

*Professor, Department of Physiology, University of Chicago, Chicago, Illinois.

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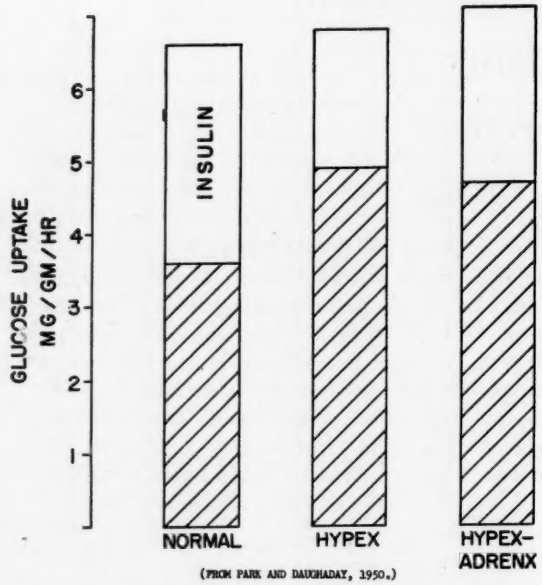

FIG. 1. Glucose uptake by diaphragms from normal, hypophysectomized, or hypophysectomized - adrenalectomized rats. The height of the cross-hatched area represents the rate without added insulin; the total height represents the rate with 0.1 unit insulin per ml. of incubation fluid. The data are from Park and co-workers.[20, 40]

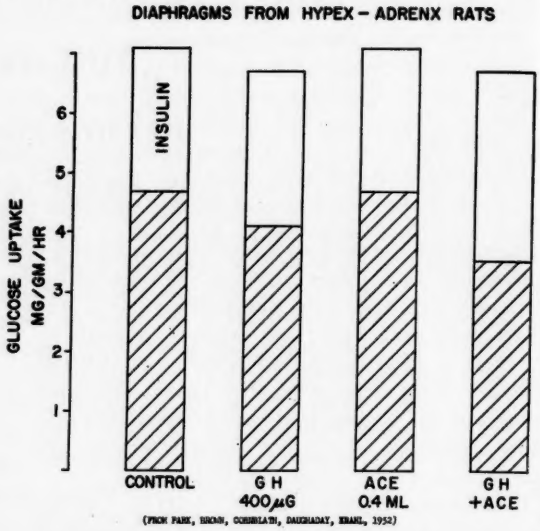

FIG. 2. Glucose uptake by diaphragms from hypophysectomized-adrenalectomized rats after injection of growth hormone (G.H.) and lipoadrenal extract, Upjohn (ACE). The growth hormone was given 24 hours, the lipoadrenal extract in divided doses 24 and 6 hours, prior to diaphragm removal. The data are from Park, Brown, Cornblath, Daughaday, and Krahl.[20]

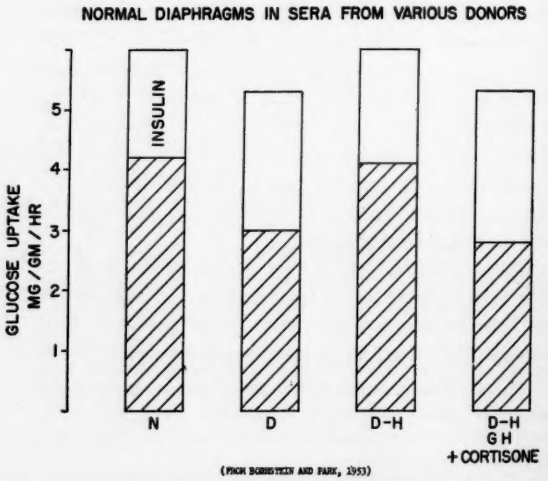

FIG. 3. Glucose uptake by normal diaphragms in sera from normal (N), diabetic (D), or diabetic-hypophysectomized (D-H) rats, and diabetic-hypophysectomized rats injected with growth hormone and cortisone. In the latter cases, doses of 0.2 mg. growth hormone and 0.1 mg. cortisone were given at 48 hours, and again at 24 hours, prior to diaphragm removal. The data are from Bornstein and Park.[21]

mental evidence that the glucose uptake of muscle in absence of excess insulin is limited by at least three factors. The first type of inhibition is intrinsic to the muscle and not dependent on a continuing supply of pituitary and adrenal factors;[18, 19] this inhibitor is the one which was overcome in the muscle of the hypophysectomized-adrenalectomized animal when insulin raised the glucose uptake from 4.6 to 7.1 mg. per gm. per hour. The second inhibitor is demonstrable by injection[2, 20] of pituitary growth hormone and adrenal cortical extract (or C-11-oxysteroids) into the rat 24 hours prior to removal of the diaphragm. It reduced the glucose uptake of diaphragms from the hypophysectomized-adrenalectomized rat from 4.6 to 3.5 (figure 2). This was reversible by insulin in vitro at low doses of the pituitary and adrenal substances but became less so as the dosage of inhibitors was raised. The third type of inhibition, related perhaps to the second, is mediated by humoral factors found in diabetic serum and was actually demonstrated with normal diaphragms (figure 3). This inhibitor in diabetic serum reduced the glucose uptake of normal diaphragms from 4.2 to 2.8 mg. per gm. per hour. The inhibition disappeared after removal of the pituitary from the diabetic serum donor; it was restored by injection of growth hormone and cortisone into the diabetic-hypophysectomized serum donor, *but not by addition of the growth hormone and cortisone to the incubation medium in vitro.*

This humoral inhibitor has been localized in a lipoprotein fraction of diabetic serum.[22]

Fat Depots. Insulin added in vitro stimulated glucose uptake by adipose tissue,[19] the average stimulation ranged from 22 per cent for perinephric fat to 78 per cent for subcutaneous fat; glucose uptake by adipose tissue from diabetic rats was subnormal and was returned toward normal by addition of insulin in vitro. Stetten and Boxer[24] demonstrated that about one third of the carbohydrate eaten by the normal rat was converted to fat, and that this fat synthesis came to a virtual stop after induction of diabetes.

Liver. Effects of hormonal imbalance on carbohydrate utilization in liver are considered by other members of this symposium, particularly Dr. Baker and Dr. Levine. There are significant similarities and differences between the response of liver, on the one hand, and that of muscle or adipose tissue, on the other hand, to excess or deficiency of insulin and other hormones. New findings with respect to glucose utilization, fatty acid synthesis, and peptide synthesis in liver will now be considered.

Glucose utilization for glycogen synthesis has been reported to be stimulated in normal *rabbit* liver slices,[25] but not in normal *rat* liver slices,[3] by addition of insulin in vitro. Further work is necessary to settle the action of *excess* insulin on the liver in vitro. But there is no doubt that *deficient* insulin leads to changes in carbohydrate metabolism of the liver.[26] Glucose uptake was decreased and glucose output from endogenous sources was increased in diabetic liver slices as compared to normal (table 1). The increased glucose output was accompanied by an increase* in glucose-6-phosphatase,[27,28,42] the final enzyme concerned with output of the glucose from endogenous precursors (figure 4). These changes in glucose utilization by livers from long-term, severely diabetic, rats were not reversible by addition of insulin in vitro; the effects of adding insulin to the medium have apparently not yet been explored with liver slices from mildly diabetic rats (see peptide synthesis, below). They are reversed, i.e., glucose utilization and glucose-6-phosphatase activity were returned toward normal, by injection of insulin into the animal some hours or days prior to liver removal.[29] The decreased glucose uptake was also returned toward normal by removal of the pituitary from the diabetic animal (table 1); this effect is not due merely to a decrease in adrenal cortical function, as the glucose uptake of liver from diabetic-adrenalectomized rats was not much above the diabetic level. These experiments indicate that glucose uptake of liver is under the control of a pituitary factor whose inhibitory effects are not directly and immediately reversible by insulin. It may be that insulin acts on the pituitary to suppress formation of the factor which inhibits glucose uptake of liver.

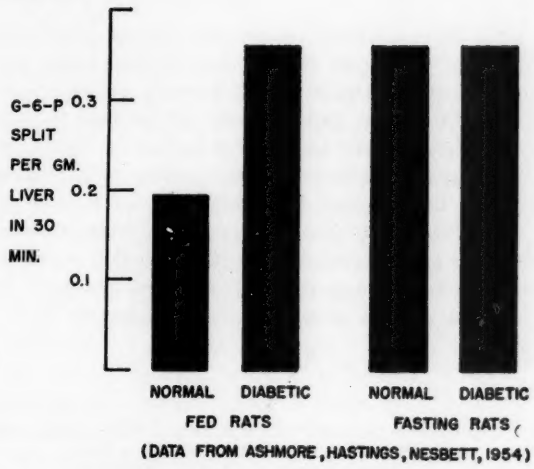

FIG. 4. Glucose-6-phosphatase activity extractable from livers of normal and diabetic rats. The amount of glucose-6-phosphate hydrolysed (ordinate) is expressed in millimoles. The data are from Ashmore, Hastings, and Nesbett.[27]

The experiments on liver which have been considered up to this point indicate first, that glucose uptake is decreased and glucose output is increased in diabetic liver; and second, that the inhibition of glucose uptake is in part due to a factor of pituitary origin. Similarly, fatty acid synthesis and peptide synthesis in liver slices are low in diabetes and restored to normal by hypophysectomy or by injection of insulin. These changes which are closely parallel to, and may be dependent on, the changes in glucose uptake will now be discussed.

GLUCOSE UTILIZATION AND FATTY ACID SYNTHESIS

Fatty acid synthesis from acetate was deficient in liver

TABLE 1

Glucose uptake and output by liver slices from fed rats. Micromoles per gm. liver per 1.5 hours (data from Renold, Teng, Nesbett, Hastings, 1953)

Rat	Glucose	
	Uptake	Output
Normal	61	69
Pancreatectomized	16	102
Diabetic, alloxan	29	149
Diabetic-hypox.	44	81
Hypophysectomized	49	54

*It is suggested that the activity of the enzyme glucose-6-phosphatase may respond to the adenosine triphosphate (ATP) level in the liver in a manner opposite to that of liver phosphorylase, the glucose-6-phosphatase being *inactivated* when the ATP level is high, while phosphorylase is *activated* by ATP and a kinase.[41]

slices from pancreatectomized cats but was normal in liver slices from the Houssay cat;[31] the inhibition was restored by injection of growth hormone into the Houssay cat (table 2). Deficient fatty acid synthesis in liver slices from diabetic animals was restored to normal by injecting insulin[34] into the diabetic animal or by feeding a high fructose diet[35] for some days prior to liver removal. It is clear that in longstanding, severe diabetes there is loss of some factor required for fatty acid synthesis; the nature of this factor has been considered by Masri et al.,[36] by Greenbaum,[37] and by Engel.[38]

TABLE 2

Conversion of radioactive acetate to long chain fatty acids by liver slices from fasting cats (data from Brady, Lukens and Gurin, 1951)

Cat	Micromoles acetate incorporated per 100 mg. fatty acids
Normal	2.0–8.0
Pancreatectomized	0 –0.7
Pancreatectomized-hypophysectomized	1.8–6.8
Pancreatect.-hypox. injected with growth hormone	0 –0.4

TABLE 3

Radioactivity of glycine-1-C^{14} incorporated into protein or glutathione by diaphragms or liver slices from diabetic rats at various times after subcutaneous administration of insulin (data are from Krahl, 1955)

Time after insulin admin.	Radioactivity incorporated, C.P.M. per mg. Diaphragm protein fraction	Liver cuprous glutathione
Hours		
no insulin	21	598
0.5	42	690
2	93	760
4	93	1,170
10	207	–
18	108	–

TABLE 4

Glycine-1-C^{14} incorporated into glutathione by liver slices from diabetic rats: effect of glucose and insulin (data are from Krahl, 1953)

Concentration glucose added to medium	Radioactivity incorporated, C.P.M. per mg. No insulin added to medium	Insulin added to medium
Mg. per cent		
0	632	598
140	1,480	2,940

GLUCOSE UTILIZATION AND PEPTIDE SYNTHESIS

Peptide synthesis was deficient in liver slices from severely diabetic rats. This was restored to normal under the same circumstances as glucose uptake and fatty acid synthesis, i.e., by injection of insulin (table 3) or by hypophysectomy prior to preparation of liver slices.[32, 33] When the liver slices were prepared from rats having diabetes of relatively short standing (3 to 4 days), it was found[32, 33] that peptide synthesis could be raised toward the normal level by addition of insulin and glucose, but not insulin alone, to the incubation medium in vitro (table 4); glucose alone had some normalizing effect.

These experiments have a number of implications. First, the initial defect in peptide synthesis in diabetes is failure of glucose uptake for energy supply; this is corrected by addition of sufficient insulin to permit normal glucose utilization. Second, as the diabetes becomes more protracted and severe, secondary changes occur, the capacity to synthesize peptides being greatly decreased. This decrease parallels the *decrease* in glucose utilization and in fat synthesizing capacity, and the *increase* in glucose-6-phosphatase under the same circumstances. These secondary changes cannot be corrected by addition of insulin in vitro. They are all corrected, however, by injection of insulin into the intact diabetic animal 6 to 24 hours or more prior to preparation of the liver slices. The biochemical nature of this long-term insulin effect has been discussed elsewhere.[33] Not all effects of insulin on peptide synthesis can yet be correlated with an action of insulin on glucose uptake.[23]

It is not possible to consider here the in vitro effects of epinephrine and glucagon on the metabolism of muscle and liver in vitro. These have been reviewed by Sutherland.[39]

SUMMARY

Imbalance in the supply of pancreatic, pituitary, and adrenal hormones induces changes in carbohydrate utilization by muscle, adipose tissue, and liver. There are changes in fatty acid synthesis and peptide synthesis which are correlated to a high degree, especially in liver, with the changes in glucose uptake.

1. Glucose uptake of muscle from diabetic animals is below normal, that of muscle from animals hypophysectomized 10 to 30 days above normal, and that of muscle from adrenalectomized animals slightly above normal. Addition of insulin to the incubation medium produces an increase in glucose uptake of muscle from normal, diabetic, hypophysectomized, adrenalectomized, and hypophysectomized-adrenalectomized rats.

There is evidence that glucose uptake in muscle is subject to at least three types of inhibition: one intrinsic to the muscle, a second induced in the muscle by pituitary

and adrenal factors, and a third of a humoral nature. The latter has been found in the lipoprotein fraction of diabetic plasma.

2. Glucose uptake of adipose tissue is below normal in diabetes. Addition of insulin to the incubation medium produces an increase in glucose uptake of adipose tissue from both normal and diabetic animals.

3. Glucose uptake of liver from diabetic animals is below normal. It is raised toward the normal level by hypophysectomy or by injection of insulin at suitable times prior to removal of liver for the in vitro measurements. Whether or not insulin has a direct action on normal or diabetic liver slices when added to the incubation medium in vitro is still unsettled and appears to depend on the previous history, and perhaps the species, of the animal used as liver donor.

Glucose output of liver from diabetic animals is above normal.

4. The rates of fatty acid synthesis and peptide synthesis are below normal in livers from diabetic animals. In both instances the rates are raised toward the normal level by the same procedures which tend to restore glucose uptake to normal, i.e., by hypophysectomy or by injection of insulin at suitable times prior to liver removal. There is evidence that peptide synthesis in liver slices from rats having diabetes of short duration can be directly stimulated by addition of insulin and glucose, but not insulin alone, to the incubation medium in vitro.

SUMMARIO IN INTERLINGUA

Observationes in Vitro de Relationes Endocrin in le Metabolismo de Hydratos de Carbon

Le nonbalanciate provision de hormones pancreatic, pituitari, e adrenal induce alterationes del utilisation de hydratos de carbon per musculos, histos adipose, e le hepate. Il ha alterationes in le synthese de acido grasse e de peptido, correlationate—specialmente in le hepate—con le alterationes del absorption de glucosa.

1. Le absorption de glucosa per musculos ab animales diabetic es infranormal; illo per musculos ab animales 10 a 30 dies post hypophysectomia es supranormal; e illo per musculos ab animales adrenalectomisate es levemente supranormal. Le addition de insulina al medio de incubation produce un augmentate absorption de glucosa in musculos ab rattos normal, diabetic, hypophysectomisate, adrenalectomisate, e hypophysadrenalectomisate.

Il ha datos a supportar le conception que le absorption de glucosa per musculos depende de al minus tres typos de inhibition: un prime que es intrinsec al musculo, un secunde que es inducite in le musculos per factores pituitari e adrenal, e un tertie que es de natura humoral. Le tertie ha essite constatate in le fraction lipoproteinic de plasma diabetic.

2. Le absorption de glucosa per histos adipose es infranormal in diabete. Le addition de insulina al medic de incubation produce un augmento del absorption de glucosa per histos adipose ab animales tanto normal como diabetic.

3. Le absorption de glucosa per le hepate de animales diabetic es infranormal. Illo es augmentate a nivellos normal per hypophysectomia o per le injection de insulina a un certe periodo ante le ablation del hepate pro le mesurationes in vitro. Il remane indecise si insulina ha un effecto directe super normal o diabetic sectiones de hepate quando illo es addite in vitro al medio de incubation. Il pare que le responsa a iste question depende del historia precedente e possibilemente del specie del animal que es usate como donator de hepate.

Le discarga de glucosa per le hepate ab animales diabetic es supranormal.

4. Le synthese de acido grasse e de peptido in le hepate ab animales diabetic es infranormal. In ambe casos le synthese es augmentate verso nivellos normal per le mesme mesuras que tende a restaurar le norma del absorption de glucosa, i.e., per hypophysectomia o per le injection de insulina a un certe periodo ante le ablation del hepate. Il ha factos a indicar que le synthese de peptido in sectiones hepatic ab rattos con diabete a breve duration pote esser stimulate per le addition al medio de incubation in vitro de insulina e glucosa, sed non de insulina sol.

DISCUSSION

Please see page 219 for discussions of this paper and that of Dr. Rachmiel Levine, which follows.

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The Relation of Insulin to Liver Metabolism

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Ever since the recognition of diabetes mellitus as a disease entity, considerations of interrelations of the pancreas with liver metabolism have occupied a prominent role. In Minkowski's untreated depancreatized dogs,[1, 2] and in other experimental diabetic preparations,[3, 4] it was well substantiated that in the liver the glycogen concentration rapidly became low, the fat content slowly increased,[5, 6, 7, 8] and ketone production increased.[9, 10] Early in the history of diabetes mellitus, it was therefore recognized that a deranged liver metabolism was consistently associated in some manner with the symptoms of glycosuria and hyperglycemia. While subsequent experimentation has revealed many of the correlations between hepatic function and regulation of blood glucose, the question whether impaired liver metabolism is a *direct* result of the lack of insulin remains open. While several investigators[11, 12, 13] have claimed a direct action of insulin upon glucose production by the liver, many others[14, 15, 16] have failed to find any such effect. The present paper will attempt to examine critically this large body of evidence. The earlier literature has been reviewed extensively in the monograph by de Duve.[17] The general viewpoint that will be formulated is based upon the assumption that insulin does not have a direct action upon the isolated liver, but that insulin affects hepatic metabolism only indirectly. While it is believed that the available evidence is not in conflict with such a conclusion, it is admitted that the opposite viewpoint cannot be wholly rejected at the present time.

In Minkowski's laboratory in the period between 1890 and 1900 attempts were made to delineate the role of the liver in diabetes by hepatectomy experiments. Kausch[18] demonstrated that hepatectomy led to hypoglycemia both in normal birds and in previously depancreatized birds. However, the decisive experiments came later when Mann[19, 20, 21] perfected the technic of hepatectomy in the dog and demonstrated that hepatectomy is followed by hypoglycemia in the normal as well as in the depancreatized animal. It was therefore apparent that the liver is necessary for the production of the most prominent of the signs of diabetes, namely, hyperglycemia. During this same time, it was firmly established that the glycogen levels following pancreatectomy in practically all species were low,[3, 4, 17] and ketosis[9, 10, 17] followed.

In 1928, Soskin[22] demonstrated that various agents which elicited hyperglycemia in the intact dog failed to do so in the hepatectomized animal. The previous experiments of Mann[19, 20, 21] together with those of Soskin[22, 23] clearly showed that the bulk of the blood sugar was manufactured in the liver. Parenthetically, it should be stated that a small amount of blood glucose may be derived under certain conditions from the kidney.[24, 25]

After the discovery of insulin and its subsequent application therapeutically and experimentally, it was anticipated that insulin would be the central hormone which corrected the abnormal metabolic activities of the liver in diabetes. This was borne out by the experiments which demonstrated that administration of insulin to a depancreatized animal having a fatty liver, low glycogen levels, and intense ketosis corrected these impairments.[26, 27] However, the chronic fatty liver of diabetes could be corrected only by lecithin[28] or choline[29] and not by insulin. The controversy concerning the role of insulin in liver metabolism began at the same period and is still not resolved. The administration of insulin to intact animals did not lead to an increase in hepatic glycogen,[30, 31] but in fact it led in most instances to hepatic glycogenolysis. Goldblatt[33, 34] showed that in fasting rabbits under certain conditions the liver glycogen was increased by the prior administration of insulin. These experiments have not been confirmed. Fed intact rabbits, even in Goldblatt's report,[33] did not deposit liver glycogen under the influence of insulin.

Burger,[35] de Duve[17] and others[36] have drawn attention to the fact that certain commercial insulin preparations raised the blood sugar initially before hypoglycemia supervened. This was overlooked when blood samples were not analyzed within 10 or 15 minutes after the

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administration of insulin. This glycogenolytic property was at first attributed to insulin, but Abel[37] crystallized relatively pure insulin and showed that his preparation did not have a hyperglycemic effect after injection. The hyperglycemia was considered for years to be produced either by an impurity or by insulin itself. It is now well known that this hyperglycemic factor is glucagon.[17, 36] Bouckaert and de Duve[32] have justifiably urged caution in the interpretation of data which allegedly demonstrate an hepatic glycogenolytic action of insulin. Since it is known that several commercial brands of insulin at present contain, or have previously contained, glucagon, it may be that the hepatic glycogenolysis following injection of insulin[30, 31] may have resulted from glucagon included in the material administered.

Bridge[31] investigated liver-insulin interrelations in the following manner. In intact rabbits, glucose was administered intravenously to maintain the blood sugar at hyperglycemic levels. The liver and carcass glycogen levels were determined, and the oxygen consumption of the animal was measured throughout the period. He could account for approximately 50 to 60 per cent of the utilized glucose by the rise of glycogen in the various tissues, and he assumed that the remaining retained glucose was either oxidized or transformed. Injection of insulin (1 or 4 units per gram glucose administered) did not affect the oxygen consumption, but the distribution of the glycogen was consistently altered. If one unit of insulin per gram glucose administered was added to the intravenous glucose infusion, the muscle glycogen increased, while the liver had a smaller glycogen content than in the absence of the additional insulin. When 4 units of insulin per gram glucose administered were given, this phenomenon was accentuated, as can be seen from the data in table 1 taken from Bridge's paper.[31] Bridge concluded from this that while insulin under many circumstances could increase muscle glycogen in the presence of adequate amounts of blood glucose, the administration of insulin did not elevate liver glycogen. In fact, under these circumstances, the glycogen seemed to shift from the liver to the muscles while the total glycogen content remained steady. Bridge[31] took the precaution of testing whether these apparently unusual effects of insulin resulted from stimulation of the adrenals. When he repeated these experiments in adrenalectomized animals, the results were the same, and he concluded that the observed liver glycogenolysis did not result from a reflex discharge of adrenalin. Unfortunately, the insulin employed by Bridge was probably not free of hyperglycemic factor.[32]

From time to time evidence appeared which indicated a direct effect of insulin on the carbohydrate metabolism of the liver. For example, the older experiments of Issekutz,[38] Molitor and Pollak[39] on perfused livers, and Seckel[40] on liver slices, indicated an inhibition of glycogenolysis. The experiments of Taubenhaus and Soskin[41, 42] demonstrated that insulin given in vivo to dogs one to three hours before sacrifice inhibited glycogenolysis by liver brei in the presence of high sugar con-

TABLE 1

Fate of glucose infused into normal unanesthetized rabbits

Group	Number of rabbits per group	Average weight in kilos	Glucose in grams		Glucose retention grams per kilo of net weight average after 6 hours of infusion		
			Intake	Excreted in urine	Total	As liver glycogen	As muscle glycogen
B. Glucose infused at rate 1.5 gm./kg./hr. No insulin	8	2.41	22.6	1.35	10.8	3.8	2.3
C. Glucose as in B, with added 1 unit insulin per gram glucose	8	2.50	21.8	0.8	11.2	1.9	4.0
D. Glucose as in B, with added 4 units insulin per gram glucose	8	2.50	23.7	2.3	10.7	0.8	5.2

Taken from the data of Bridge.[31]

centrations. In spite of these reports which occasionally indicated a direct inhibitory effect of insulin on net glucose release by the liver, most of the experiments testing this point were negative. Lundsgaard's laboratory during the 1930's concerned itself with this problem.[43, 44, 45] The results clearly demonstrated that the perfused livers of cats and rabbits were nonresponsive to insulin added to the perfusion fluid. Under the conditions of their experiments, glycogenolytic-free insulin neither increased the rate of glucose uptake, decreased the rate of glucose production, nor inhibited the glycogenolysis induced by administration of adrenalin. These experiments were as free of technical errors as experiments with perfused organs can be, since Lundsgaard proved that the perfused liver functioned well, as measured by urea production, oxygen consumption, carbon dioxide production, utilization of various substrates, and so on. It should be noted that the rabbit was the only animal studied whose perfused liver displayed a net uptake of glucose. In the rabbit, the uptake by the liver was approximately 3.0 gm./kg./liver/hr., and this huge rate was not altered in the presence or absence of insulin.[16] Perfused livers from cats failed to have a net uptake of glucose under all conditions studied. However, while the net uptake of glucose is negative, Kruhoffer and Muntz,[46] using C^{14}-labeled glucose, proved that a small quantity was oxidized to carbon dioxide by such a perfused cat liver, giving still further evidence that the liver was functioning in a normal metabolic manner, but that glucose utilization by the liver from both normal and diabetic cats is small under the circumstances studied.

In 1940, Gemmill[47] demonstrated that insulin added in vitro to rat diaphragms increased the rate of glucose uptake and the rate of glycogen deposition. This has been repeatedly confirmed[48, 49, 50] and was extended to other skeletal muscles sufficiently thin to permit adequate diffusion of oxygen and substrates. Similar attempts to demonstrate an in vitro effect of insulin upon glucose metabolism by liver slices have failed.[15, 49] While it is well known that liver slices and homogenates respire actively for several hours and utilize many substrates, the addition of glucose and/or insulin has not been consistently shown to produce a net uptake of sugar or an inhibition of the output of sugar from such a preparation.[15, 49, 51, 52, 53] The addition of insulin (free of hypoglycemic factor) to liver slices has not been shown to alter the glucose uptake or rate of glycogenolysis. A few exceptions to this generalization exist, and some of these will be examined.

Bach and Holmes[54] demonstrated glucose uptake and glycogen deposition in liver slices from rabbits. Under certain circumstances small uptakes were registered when the glucose in the medium was between 1,000 to 2,000 mg. per 100 ml. and when the calcium in the medium was lowered. Under other circumstances neither deposition of glycogen nor glucose uptake occurred. These experiments resembled the older in vivo experiments of Goldblatt[33] in rabbits. Hastings and associates[55] initially found an in vitro effect of insulin upon rat liver. In a high potassium environment in the presence of glucose, insulin was reported to facilitate glucose uptake into glycogen by liver slices. However, Hastings and associates[15] subsequently were unable to repeat those findings.

Following the work of Wierzuchowski[56] and of Soskin and Levine[57] on the utilization of glucose in eviscerated dogs under various circumstances, Lundsgaard[58] and de Duve[59] confirmed that the rate of utilization was dependent upon the blood sugar concentration within certain limits. De Duve and others[60] in Bouckaert's laboratory reinvestigated the effect of insulin on glucose utilization of the liver by indirect means. These workers first demonstrated that the maximal effective dosage of insulin in dogs was approximately 8u/kg./hr., since the administration of higher dosages resulted in no further increase of glucose utilization. During the injection of a maximal dose of insulin, the intact dog requires about 1,500 mg./kg./hr. of glucose in order to maintain a normal blood sugar level. In hepatectomized or eviscerated animals receiving a maximally effective amount of insulin, only about 500 to 600 mg./kg./hr. of glucose is required to maintain the same normal level of blood sugar. De Duve interpreted these data as indicating that the lesser amount utilized in hepatectomized animals than in intact dogs (1,500 minus 600, or 900 mg./kg./hr.) was an index of the amount of glucose normally utilized by the liver. This would assign an enormous capacity for glucose uptake to the liver, which presumably must therefore be under the regulatory influence of insulin. This is the conclusion de Duve reaches in his book[17] and in his review written with Bouckaert[32] on the nature of insulin action.

Meanwhile, it had become evident through the work of enzyme chemists that the first metabolic transformation of glucose is the formation of glucose-6-phosphate in the presence of hexokinase and ATP. Glucokinase is present in varying concentrations in various tissues, as demonstrated in table 2, taken from a paper by Long.[61] As can be seen in table 2 the glucokinase concentration in liver tissue is the lowest of all tissues analyzed. This finding is consistent with the absence of net glucose uptake by perfused cat livers[45] and by liver slices from various species.[48, 52, 53] However, it is not

TABLE 2

Hexokinase activities of rat tissues
(Expressed as mM glucose utilized per mg. dry weight of tissue per hr.)

Tissue homogenates	– Q glucose average
Brain	27.1
Stomach	17.7
Colon	17.2
Heart	14.9
Testis	14.0
Small intestine	11.7
Cecum	10.9
Uterus	9.3
Kidney	7.5
Skeletal muscle	6.0
Spleen	6.0
Pancreas	5.9
Lung	4.3
Liver	1.4

Taken from the data of Long.[61]

consistent with de Duve's interpretation of the apparently enormous glucose uptake by the liver. The phenomenon described by de Duve[60] was reinvestigated recently in our laboratory by Lang and Goldstein[62] who demonstrated that the data of de Duve could be quantitatively confirmed. However, when glucose utilization by the hind limbs exclusively was measured by determining A-V glucose differences between the aorta and vena cava and by measuring blood flow to the area, it was observed that maximal glucose utilization of such muscle preparations was approximately 600 mg./kg./hr. in eviscerated dogs, and 1,500 mg./kg./hr. in intact animals, provided maximal amounts of insulin were infused in all experiments. This suggested that under these circumstances the liver released a humoral substance which in some manner increased glucose utilization by the periphery. There is no need to postulate that insulinization resulted in augmented glucose uptake by the liver. In fact, a consistent in vitro effect of insulin upon glucose utilization by isolated liver preparations has yet to be demonstrated. In the light of the data of Lang and others[62] the interpretation of de Duve[60] does not appear possible. It should be noted, however, that Lundsgaard[63] observed that the rate of sugar utilization with maximal insulin dosages remained the same in both intact and hepatectomized or eviscerated cats. These findings differ both from the data of de Duve[60] and of Lang and others[62] obtained in dogs, and no explanation is available to account for the apparent species difference.

In addition, the isolated liver slice or perfused liver from most species cannot be shown to take up glucose to any extent. In the experiments cited to this point, it would appear that neither insulin nor glucose directly affects the metabolism of the isolated liver. In contrast, several investigators[12, 41, 64, 65] have reported an action of insulin or glucose administration on liver metabolism in vivo. These data come mainly from two types of experiments. Soskin, Essex, Herrick and Mann[64] demonstrated by blood flow measurements and A-V differences that the hepatic glucose production in dogs could be influenced by exogenous glucose. When exogenous glucose was given to intact animals, hepatic glucose production ceased and an apparent glucose uptake ensued. After the glucose concentration had come back to its original levels, glucose production was slowly resumed. This was confirmed later by Bondy and Meyer.[66] However, the effects of insulin on this system were not studied. The experiments of Sherlock et al.[12, 67] in the past few years on man extended these earlier results. The British workers examined glucose production in normal and diabetic humans, and determined the effect of an intravenous injection of 0.1 u/kg./insulin on hepatic glucose release. Within five minutes, insulin produced a sharp drop in the glucose production, and in certain cases led to a net uptake, as calculated by Sherlock. Within approximately 30 minutes, the hepatic glucose production had again resumed its normal course. These data can be seen in table 3 taken from Sherlock.[12] These experiments were interpreted as indicating a definite effect of insulin on glucose uptake and release by the liver. In the experiments of Sherlock et al.,[12, 67, 68] the measurement of the hepatic glucose production is indirect, since the total splanchnic area is included in the measurements of the A-V glucose differences. Since the insulin-induced glucose uptake was not directly shown to take place in the liver itself, the glucose utilization could very well have occurred in the gastrointestinal area of the splanchnic bed,

TABLE 3

Effects of insulin on hepatic glucose output by normal and diabetic human subjects

Time after insulin administration in minutes	Average hepatic glucose output mg. per square meter per minute	
	Normal	Diabetic
Before insulin	125	140
0	125	140
5	50	10
15	10	10
30	70	40
45	205	40
60	150	65
80	125	110

These data are taken from figures 8 and 9 of the paper by Sherlock et al.[12]

where many fat depots are present. In this regard, Hausberger et al.[69] have shown that lipogenesis in adipose tissue is markedly increased after insulin administration, but the time-response curve has not yet been established. However, the experiments[64, 66] showing a direct effect of blood glucose levels on hepatic glucose production were performed in such a manner that interpretation is unequivocal. Furthermore, since a few of Sherlock's diabetic patients showed a negative A-V hepatic glucose difference for a short time interval after insulin administration, it appears that both insulin and glucose under certain conditions have an inhibitory effect on the net release of sugar from the liver. Recently Bondy[70] has questioned the general validity of the hepatic vein catheterization technic, but it is unlikely that this criticism affects the above results qualitatively.

Another group of experiments has recently been performed on the effects of insulin on the metabolism of C^{14}-labeled glucose in isolated liver slices. While net uptake of glucose is small or negligible in these preparations, Hastings and co-workers[15, 71] have shown that labeled glucose appears in liver glycogen and in respiratory carbon dioxide. Initially Hastings et al.[55] published positive effects of insulin in vitro on the incorporation of labeled glucose into glycogen. However, this could not be consistently repeated, and a recent paper from Hastings' laboratory denies the effect. These workers[15] state the following unequivocal summary of their attempt to demonstrate an action of insulin on glucose metabolism by liver slices.

Many attempts to demonstrate an effect of insulin on hepatic carbohydrate metabolism in vitro have been made in this laboratory. Although it was thought in one series of experiments that insulin, in vitro, had a demonstrable effect on glucose uptake and glucose output, an extension of the series has failed to confirm this preliminary conclusion. Indeed, as of the present time, we have been unable to establish a consistent, reproducible effect of insulin in vitro, and therefore no detailed account of these experiments will be given. In brief, insulin has been added in concentrations of up to 1 u/ml., and to insure good initial penetration of the hormone, whole liver and single lobes were on occasion perfused with insulin solutions before slicing. Measurements made included uptake of glucose, fructose, and pyruvate, as well as glycogen deposition and CO_2 production from these substrates, total glucose output, glucose production from fructose and pyruvate, and total glucose phosphorylation. Insulin was added to liver slices from normal, from both mildly and severely diabetic, and from adrenalectomized, hypophysectomized, and diabetic-hypophysectomized rats. In no instance was a consistently positive effect obtained.[15]

From the data provided by Kruhoffer and Muntz[46] from Lundsgaard's laboratory on the fate of C^{14} glucose in the perfused cat liver, it can be shown that the net uptake of glucose would account for a very small percentage of the glucose turnover or the carbon dioxide production. Fructose, under the same circumstances, is at least 10 to 20 times as actively metabolized by the perfused cat liver, and a net glycogen formation from fructose can be demonstrated.[46] It is obvious that the magnitude of the C^{14} glucose uptake is an inappreciable fraction of the total carbohydrate turnover of the liver.

Since the work of Houssay,[72, 73] Long,[74] Evans[75] and many others, it has been realized that the frank expression of the diabetic state, namely hyperglycemia, glycosuria, ketosis, etc., was due not only to the absence of insulin but also to the simultaneous presence in the depancreatized animal of an intact pituitary, adrenal cortex and thyroid. The possibility arises that the disturbed metabolism of the liver in the depancreatized animal or in the diabetic human does not result primarily from a lack of insulin, but from the disturbed endocrine balance when the pituitary-adrenal cortical axis is predominant. This is consistent with the viewpoint expressed by several workers in this field.[48, 76, 77, 83]

In experiments reported by Renold et al.[15] insulin was administered in vivo to alloxanized diabetic rats, and these animals were sacrificed at varying time intervals after receiving therapy. Samples of muscle and of liver were removed, and various metabolic reactions were studied, employing C^{14}-labeled glucose, pyruvate, fructose and acetate. The rate of incorporation of the marked glucose into glycogen by these tissues was examined. In the skeletal muscle slices from rats sacrificed ten minutes after the initial administration of insulin, there was a definite increase in glucose uptake. This effect remained high for approximately an hour or less, and then began to decrease. In contrast, no such effects could be seen in liver slices prepared from these same animals during the first several hours. Only after 6 to 12 hours could significant increases in hexokinase activity and conversion rates of pyruvates to fatty acids be obtained, and these increases became greater than normal levels after approximately 24 hours. These data are summarized in tables 4 and 5 taken from the paper by Renold et al.[15] Similar time relations were observed with other metabolic systems, and it was consistently found that restoration of normal liver metabolic function took much longer than restoration of normal metabolic function in muscle. From this, the authors concluded that the effect of insulin on the uptake of sugar by the liver and oxidation to CO_2 was indirect, but that the action on the periphery was

a direct one. It was postulated that the indirect effect took a long time because of probable intermediation of peripheral factors, which exhibited subsequent effects upon the liver. These peripheral factors are, at the present moment, unknown. The possibility exists that peripheral factors are produced in those tissues which are definitely insulin sensitive (e.g., muscle and the connective fat tissue cells), and these factors then affect the liver. A second possibility is that insulin exerts a direct inhibitory action on the anterior pituitary, thereby making the animal, for a certain period, hypopituitary in effect. According to Renold et al.,[15] a third possibility would involve the direct but slow effect of insulin on synthesis of liver enzyme systems. The indirect effect of insulin on liver and the lack of the direct effect was also demonstrated by the extensive work of Chaikoff and his laboratory[78, 79, 80, 81] on lipogenesis in liver slices (from many substrates) in normal, fasted and diabetic animals. It was demonstrated that fasting, the feeding of low carbohydrate diets, or alloxanization produced a tremendous decrease in the rate of lipogenesis by liver slices from glucose, acetate and other precursors. In a depancreatized or alloxanized animal, the prior prolonged feeding with fructose, which probably provided needed concentrations of certain members of the glycolytic cycle essential in lipogenesis, restored lipogenesis to normal in the complete absence of insulin.[80] Lukens, Gurin, and Brady[82] have demonstrated that the removal of the hypophysis or adrenals in cats restored the previously very low glycogenetic and lipogenic activity of liver slices of depancreatized cats. Furthermore, the "normal" in vitro rate of lipogenesis displayed by livers from hypophysectomized, pancreatectomized cats could be lowered by the in vivo injection of cortisone or anterior pituitary extract. Lukens[83] remarks that this points to the direct action of the contra-insulin hormones, rather than to any direct

TABLE 4

Measurements of total glucose phosphorylation by rat liver slices at varying time intervals after insulin administration

Number of experiments	Animals	Total glucose phosphorylation (percentage of normal)
7	Normal	100± 6.2
7	Diabetic	6± 1.8
7	Diabetic, given insulin intravenously from 10 to 70 minutes before sacrifice.	7± 2.1
5	Diabetic, given insulin intravenously and subcutaneously for 24 to 72 hours before death.	87±16.3

These data are taken from a paper by Renold et al.[15]

TABLE 5

Glycogen deposition from glucose by rat liver slices and by rat diaphragms at varying intervals after insulin administration

Experiment number	Animals	Duration of insulin administration	Glycogen from glucose—uMoles glucose equivalents per gram net weight per 90 minutes	
			Liver	Diaphragms
	Normal	Minutes		
1	"	0	22.2	4.0
2	"	0	17.4	5.2
3	"	0	14.0	6.7
	Diabetic	Minutes		
4	"	0	0.1	2.8
5	"	0	0.1	3.5
6	"	0	0.1	4.8
	Diabetic	Minutes		
7	"	10	0.1	20.2
8	"	10	0.1	14.6
	Diabetic	Hours		
11	"	1	0.2	21.4
12	"	6	0.5	2.0
13	"	6	0.4	10.0
14	"	24	12.5	3.0
15	"	48	23.4	3.2
16	"	48	28.0	3.2

These data are taken from a paper by Renold et al.[15]

effect that insulin may have, on glycogenesis or lipogenesis in liver. It therefore appears that both the decreased hepatic fat synthesis and the low hepatic glycogen levels found in the absence of insulin are not under the direct mediation of insulin, but are instead a derivative effect of insulin action, dependent upon extrahepatic influences probably resulting primarily from a relative pituitary-adrenal cortical excess.

A body of papers exist which demonstrate a direct in vitro action of insulin on lipogenesis by liver slices from normal fed animals,[84, 85, 86] and in one instance, from liver slices of fasting rats.[87] However, Gurin,[88] Bloch,[84] and Weinhouse[86] have all reported consistently negative effects of insulin on liver slices from fasted or diabetic rats. The significance of the positive effects in liver slices and in the perfused liver[89] from intact animals is not clear.* However, it should be noted that the rise in lipogenesis elicited with insulin in vitro is not nearly as great as that achieved by in vivo administration of insulin.[81]

Explanations for the lack of effects of in vitro insulin upon glucose metabolism by livers from diabetic preparations must consider possible changes in the "set" of adaptive enzymes which occur in the livers of diabetic or starved animals. For example, it has recently been shown[90, 91, 92] that glucose-6-phosphatase activity is enhanced in livers from such animals. If this increase were extensive, then no net glucose uptake could occur, because glucose-6-phosphate would be dephosphorylated as soon as the hexokinase reaction had carried glucose to glucose-6-phosphate. The relative or absolute increase in glucose-6-phosphatase activity could conceivably either induce, or be a subsequent "adaptation" to, the enhanced gluconeogenesis which is found in diabetes. However, if this enzyme level were the controlling factor regulated by insulin, it must be postulated that the rapid effects observed by Sherlock et al.[12] of insulin on decreased hepatic glucose output are associated with a similarly rapid effect of insulin on glucose-6-phosphatase activity. Yet, it has recently been reported that the time required for restoration of hepatic glucose-6-phosphatase activity in alloxan diabetic rats after injection of insulin is in the order of magnitude of several hours.[92] It therefore appears that glucose-6-phosphatase levels could not be a primary focus of possible direct insulin action on the liver. Another possibility must include consideration of insulinase levels in the liver. Mirsky et al.[93, 94, 95] and others,[96] have recently shown that insulin is rapidly destroyed by the liver. In most previous in vitro experiments, adequate precautions to prevent inactivation of insulin have not been taken. With the use of insulinase inhibitors now available[95] these in vitro experiments can be repeated to explore this possibility.

Nevertheless, a large body of apparently technically flawless evidence exists which fails to demonstrate a direct action of insulin on isolated liver systems, from homogenates to perfused livers, where many different metabolic systems have been studied. Under certain conditions, an action has been suggested, and this body of evidence is admirably summarized in a recent review by Krahl.[97] Certain in vivo evidence, already outlined, has indicated that insulin administration is almost immediately accompanied by a diminution of glucose release by the liver. However, after insulin administration to diabetic animals, restoration of normal rates of hepatic protein synthesis;[97] levels of glucose-6-phosphatase;[92] glucose uptake;[15] and lipogenesis[79] requires a latent period of several hours. In contrast, insulin administration to alloxan diabetic rats is followed within minutes by an augmentation of glucose uptake by muscle.[15]

From the available evidence, which unequivocally demonstrates an intimate relationship between insulin and hepatic metabolism, the following working hypothesis is postulated to attempt to account for some of the apparent discrepancies of interpretation of the effects of insulin on liver.

Insulin and liver metabolism are intimately but indirectly interrelated via mediating influences, which are capable of directly affecting hepatic glucose metabolism. The nature of these "mediating influences" must at the present time include two broad categories. In the first case, the lack of insulin directly creates an endocrine imbalance which favors a marked domination of anterior pituitary effects. The products of anterior pituitary stimulation then must be shown capable of inhibiting hepatic glycogenesis, lipogenesis, and net glucose uptake by the liver. Evidence that administration of adrenal cortical steroids and anterior pituitary hormones may be associated with these changes under certain conditions is in the literature.[76, 82, 83] Once such an endocrine imbalance has been established, the administration of insulin would require a latent period to restore the enzymatic machinery which has been changed in the liver by these conditions. Therefore, it would not be possible to demonstrate an hepatic in vitro effect of insulin, but only an in vivo effect after a certain time interval. This

*Recently, Haft and Miller[89a] have published a preliminary note indicating that the addition of insulin in relatively large amounts to the perfusion fluid resulted in a restoration of impaired lipogenesis from C^{14}-labeled acetate by perfused livers obtained from alloxan diabetic rats not previously treated with insulin. However, these effects were obtained only if ketogenesis was not pronounced. Full evaluation of these data must await the publication of complete details of this provocative report, which it is hoped will be forthcoming soon.

explanation takes into account the various examples of restoration of impaired liver metabolic functions in diabetes following hypophysectomy.

However, this explanation does not account for the rapid decrease in net hepatic glucose release in man following injection of insulin (Sherlock et al.[12]). It should be pointed out that recently Henderson et al.,[65] measuring the rate of dilution of C^{14} glucose administered intravenously to pancreatectomized dogs, found that injection of insulin was followed by a rapid enhancement of glucose utilization, but that hepatic glucose production was not diminished until somewhat later. Maximal depression of hepatic glucose release occurred slowly, and the inhibited glucose appearance was most pronounced after the total increased utilization effect was over. This failure of quantitative confirmation of the conclusions of Sherlock et al.[12] might be attributed to one of two sets of differences existing between the work of the two laboratories. Perhaps dog livers respond differently to insulin than do human livers. Secondly, the two different sets of methods may be measuring different things, even though both sets of results have been interpreted to indicate changes in hepatic glucose production. The Canadian laboratory's findings are compatible with the large body of data previously cited which indicate that hepatic effects are secondary to the action of insulin on the transfer of glucose into certain peripheral cells. It is not inconceivable that liver cells of man uniquely respond to insulin in the same manner that muscle cells of all mammals do, namely by having a diminished barrier to the entry of glucose. If this were the case, then the data of Sherlock et al.[12] could be interpreted as an apparent net decreased hepatic glucose release after insulin resulting from an increased uptake. This would be compatible with the speed of the observed changes. In contrast, it is known that perfused livers of cats, dogs, and rabbits are nonresponsive to insulin added to the perfusing media,[16, 43, 44, 45] and therefore it has been assumed that glucose uptake by the liver of these animals is not under insulin regulation. Since the findings of Sherlock et al.[12] are the only published data that we are aware of which seriously challenge the hypothesis that the physiologic effect of insulin on hepatic metabolism is mediated indirectly via extrahepatic, insulin-responsive influences, it is of great importance to establish if Sherlock's findings can be confirmed under similar experimental conditions in dogs, cats, rabbits, or rats. If Sherlock's work can be confirmed in these species, it must then be established that the splanchnic area is not contributing to the apparent insulin effect on hepatic glucose release before it can be definitively stated that insulin has a direct effect on hepatic glucose production.

In the second category of mediating influences, it is necessary to postulate the existence of some extrahepatic humoral factor which is released in response to insulin, and which alters various hepatic metabolic processes in some manner other than by simply opposing pituitary effects.

If the "primary" action of insulin—namely its effect upon glucose transfer into certain cells[98]—is the only direct action of insulin, then the postulated humoral factor must be derived from the responsive cells at the time and site of action. In muscle, there are several known materials present in relatively high concentrations whose physiological effects are unknown. The effects of insulin in vitro on the release of anserine, carnosine, carnitine, myokinin, etc., from muscle are unknown, and the action of these materials on hepatic glucose production also remains unknown. It is well established that hepatic glucose release must vary under different physiological conditions. For example, the steady concentration of blood glucose during exercise or work could result only from an increased hepatic production; and a decreased glucose output following high glucose levels, as occurs after meals, also undoubtedly takes place. Insulin seems to be required to help "set" the particular blood sugar concentration which is to be kept steady under these varying circumstances.

A definitive conclusion as to whether insulin does or does not exert a direct effect upon the metabolism of the liver cell cannot be made with assurance at present. We believe, however, that the bulk of available evidence makes it imperative that all possible indirect actions be explored. The viewpoint presented here is similar to the opinions expressed in the reports of Lundsgaard[16, 58] and Hastings.[15]

SUMMARY

A survey of the literature has clearly indicated the close relationship of insulin to liver metabolism. The derangements in hepatic fat, carbohydrate, and protein metabolism which are found in both experimental and clinical diabetes are briefly described. These disturbances can be rectified, after varying time periods, by the in vivo administration of insulin. However, addition of insulin in vitro to isolated perfused livers, to liver slices, to homogenates, and to particle-free extracts from diabetic animals has rarely and inconsistently resulted in any correction of these metabolic impairments. This is in marked contrast to the consistent in vitro effects of insulin on glucose uptake by perfused hind limbs or diaphragms. Furthermore, various workers have demonstrated that the time required after the in vivo administration of insulin to diabetic animals for correction of

various hepatic metabolic disturbances is much longer than that required for the insulin effects on glucose uptake and glycogen formation by muscle. From these and other data which are summarized, it has been tentatively concluded that the action of insulin on hepatic metabolism is mediated indirectly, possibly via direct or indirect interrelations with the pituitary; and/or through an effect on the release of a hypothetical substance from a peripheral tissue directly responsive to insulin.

SUMMARIO IN INTERLINGUA

Le Relation Inter Insulina e le Metabolismo Hepatic

Un revista del litteratura ha indicate clarmente le existentia de un relation intime de insulina al metabolismo hepatic. Es presentate un breve description del disrangiamentos in le metabolismo de grassia hepatic, de hydratos de carbon, e de proteina que es incontrate in diabete e clinic e experimental. Iste disordines pote esser rectificate, post varie intervallos de tempore, per le administration in vivo de insulina. Tamen, le addition in vitro de insulina a isolate hepates perfundite, a sectiones hepatic, a homogenatos, e a extractos (sin particulas) ab animales diabetic ha resultate rarmente e pauco uniformemente in ulle correction del mentionate defectos metabolic. Iste facto contrasta marcatemente con le uniforme effectos in vitro que insulina exerce super le acceptation de glucosa per perfundite extremitates posterior o diaphragmas. In plus, varie investigatores ha demonstrate que le tempore requirite post le administration in vivo de insulina a animales diabetic usque al correction de varie disturbationes del metabolismo hepatic es multo plus longe que le tempore requirite per le effectos de insulina super le acceptation de glucosa e le formation de glycogeno in le musculo. Iste observationes—insimul con altere datos que es summarisate in le presente articulo—supporta le conclusion preliminari que le action de insulina super le metabolismo hepatic es mediate indirectemente, forsan via directe o indirecte relationes con le glandula pituitari, e/o via un effecto super le liberation de un substantia hypothetic ab un texito peripheric que responde directemente a insulina.

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DISCUSSION OF THE TWO PRECEDING PAPERS BY M. E. KRAHL, PH.D., AND BY RACHMIEL LEVINE, M.D., AND IRVING B. FRITZ, PH.D.

DEWITT STETTEN, JR., M.D., (*New York*): I have noted, both in our own laboratory and in the presentations of two of the speakers this morning, reference made to something called the "diabetic liver slice." Curiously, one never talks about the adrenalectomized liver slice, and I believe that the danger of this nomenclature is more than a semantic one. If by diabetes or experimental diabetes we mean the removal of the normal source of supply of insulin, then, clearly, every liver slice, indeed every slice except the pancreas slice, is necessarily diabetic, and the only difference between the liver slice from a diabetic animal and the liver slice from a nondiabetic animal is one of time, one of duration of insulin lack.

Undoubtedly, as has been mentioned by several speakers, particularly by Dr. Krahl and Dr. Levine, just

preceding, there are many changes which occur in the composition of the liver incident to pancreatectomy or alloxan poisoning. Among those mentioned were changes in the composition with respect to glycogen, with respect to fat, and the more recently observed and very interesting change in the content, or at least the activity, of glucose-6-phosphatase. Many of the changes which are observed in slices derived from the livers of diabetic animals are perhaps attributable to these changes in constitution and are perhaps, therefore, only secondarily related to the preceding history of insulin supply or lack thereof.

I should like to mention in passing that Dr. Levine was meticulously careful, and throughout his entire presentation not once did he refer to a diabetic liver slice or a diabetic diaphragm.

Related to this point, I think, are the hazards implicit in drawing conclusions in regard to rates of body processes based upon experiments performed on isolated tissues, and this is perhaps particularly true in the case of the liver. Certainly the liver sliced is nutritionally in an environment which is quite foreign to it, and it may be that our failure to observe changes in sliced liver in response to one or another stimulus is due to the fact that the rate-determining step in the liver slice becomes the transfer of nutrient into the polygonal cell, a process which must be extremely slow in the sliced liver compared to that in the intact liver, wherein, as I understand it, each polygonal cell is in contact with a venous sinus through which blood is pouring rapidly. This situation certainly is not simulated by the liver slice sloshing rather leisurely in a vessel.

One other point which I should like to make, because it interested me during the morning, is in regard to the question of a one-bloc insulin effect, or what I had earlier called a unitarian hypothesis of insulin action. I think that several of the speakers this morning are inclined to favor such a hypothesis. Dr. Baker indicated—and, I think, correctly—that this hypothesis does not have at the present time the dignity of a theory. Perhaps the discrepancy, if there is one, can be resolved by pointing out differences between a hypothesis and a theory, which some of us like to preserve.

The value of a hypothesis is not determined by its ultimate truth or falsity. In my opinion, at least, the value of a hypothesis is determined by the number of useful experiments which it suggests. Certainly the unitarian hypothesis in this regard has been quite successful. The ultimate truth or falsity must remain for the future to determine.

The selection of such a unitarian hypothesis of insulin function is based, I think, in most people's minds upon the fact that it is the simplest hypothesis that can be constructed today that is compatible with many of the facts. There are, obviously, many defects observed in the diabetic which cannot be explained today either by this hypothesis or, as far as I am aware, by any other. I know of no cause-and-effect sequence which will relate the failure of tissues to utilize glucose, on the one hand, and the development of capillary aneurysms in the retina, on the other. But for the time being the unitarian hypothesis still appeals to me and, I believe, to others as a very useful hypothesis.

It is clear that the trouble in which diabetic patients and diabetic animals find themselves is not entirely attributable to insulin lack. Indeed we have heard today a good bit about agents which appear to oppose the action of insulin in one regard or another. It has been a matter of interest in our own laboratory to explore at least in one situation the nature of the agents that actually do so operate. The situation which we have studied is that of severe diabetic acidosis.

It is a clinical truism that many patients in severe acidosis require and, perhaps what is more important, tolerate doses of insulin which these same individuals would not tolerate a few days earlier or a few days later. This suggested that insulin is being destroyed excessively rapidly in the bodies of such individuals at those times when they are in acidosis. Earlier studies by Stadie, and particularly by his colleagues Marsh and Haugaard, had explored patients with chronic insulin resistance and had found in the blood stream a demonstrable antagonist of insulin, an agent which when mixed with insulin diminished or abolished the response expected in the rat diaphragm with respect to glycogen synthesis. It had been shown in Stadie's laboratory, employing technics introduced initially, I believe, by Gemmill, that in response to pretreatment with insulin, the rat diaphragm on incubation in a glucose medium accumulates more glycogen than does a control hemidiaphragm not pretreated with insulin.

Sera of normal and diabetic patients have been treated, on the one hand, with insulin and, on the other hand, with parallel amounts of buffer, and into each of these sera hemidiaphragms have been dipped, rinsed, and then incubated in the procedure which Stadie has described. At the end of an appropriate period of time the glycogen content of each hemidiaphragm is determined.

Table 1 represents observations on normal sera in which the insulin effect is represented, as in Stadie's earlier publications, as the extra glycogen deposited in the diaphragm in response to 0.2 units of insulin in the pretreatment phase. Values in quite good accord with those published by Stadie have been obtained in our laboratory.

In table 2 are shown similar data secured when the admission blood of patients in diabetic ketosis is similarly studied. It will be observed that in these four patients the insulin effect, normally of the order of 5 micrograms of glucose per gram of diaphragm, is either abolished or diminished. This finding has been reproduced now in six out of eight patients. In two patients there was no demonstrable inhibition of insulin effect. These two patients, it turned out, were not remarkably insulin resistant clinically, requiring 200 and 230 units of insulin in the first 24 hours of their treatment after hospitalization.

Shortly after treatment is instituted, evidence of this accrual insulin antagonist disappears. In two or three days after institution of treatment, the plasma again is devoid of demonstrable insulin antagonist when tested by this method, and more recently we have seen one case in which 10 hours after the institution of treatment insulin inhibitor activity could no longer be demonstrated.

Table 3 represents a study on a patient's blood, supplied to us by Dr. Mulholland. This was a woman who received 21,000 units of insulin during 24 hours after admission in profound diabetic coma from which she died. During this time her blood sugar was not observed to fall to normal levels. She was apparently almost completely insulin resistant, and at least a partial explanation for this insulin resistance is to be found in the presence of this insulin antagonist, demonstrable in her sera at dilutions, certainly, of 1 to 40 of her sera, and possibly even at dilution of 1 to 200. Computations from these data have led us to an estimate that circulating in this woman's blood was enough inhibitor to abolish the effect of approximately 10,000 units of insulin administered at one time, and it is therefore not surprising that she failed to respond to insulin administered at the rate of approximately 1,000 units an hour.

Naturally, we are endeavoring to pursue this problem in order to ascertain the chemical nature of the material with which we are dealing. We believe that it is probably not a material of adrenocortical origin. This activity cannot be simulated by the addition of a compound F to blood. It cannot be simulated by the injection of rather large amounts of ACTH into the body of the patient over a period of 48 hours. In certain individuals who analytically were found to have elevated levels of compound F in their blood, this activity was also lacking. The material so far has been shown to be a nondialyzable and relatively thermostabile. It migrates electrophoretically, mixed with the globulins of the plasma. Further information is now being obtained on the nature of this activity. There are obviously a number of possibilities which have to be either ruled in or ruled out by subsequent study.

TABLE 1

The insulin effect using sera from normal subjects and accumulation of glycogen in the rat hemidiaphragm

	No. of determinations	Insulin effect uM. glucose/gm. tissue
HAR	8	5.49
TO	2	4.59
FI	3	5.34
HAS	3	6.45
PY	4	5.39
	Average	5.45

TABLE 2

Abolition of the insulin effect by the serum of diabetics in ketosis and restitution after recovery

	Insulin effect uM. glucose/gm. tissue	Insulin effect
PO	− .56 (4)*	6.59 (3)
MU	.79 (3)	6.25 (3)
LA	3.09 (5)	6.54 (3)
BA	.75 (3)	6.60 (3)

*Number of determinations in parenthesis.

TABLE 3

Dilution studies of the serum of a patient in profound insulin resistant ketosis

Source	Dilution	Insulin effect
Case VII		
6-14-55	1:1	−2.62 (3)*
6-14-55	1:3	−1.44 (3)
6-14-55	1:9	.39 (3)
6-14-55	1:40	1.03 (3)
6-14-55	1:200	2.89 (3)
6-14-55	1:3 heated	.65 (3)

*Number of experiments.

CHAIRMAN MARKS: Dr. Stadie is here, and I think it would be of greater interest if we could prolong the proceedings just a short time to hear Dr. Stadie comment on these two papers.

WILLIAM STADIE, M.D., (*Philadelphia*): I would like to add one point to Dr. Stetten's discussion of insulin resistance. In our laboratory we subjected diaphragms from normal rats to equilibration for a variable period of time in sera from insulin resistant cases. These pretreated diaphragms were then tested for their ability to respond to insulin when equilibrated in vitro with glucose and added insulin. We found no alteration in the ability of these diaphragms to synthesize extra glycogen in response to insulin action. In other words, the resistant sera appeared to produce no permanent action upon the diaphragm when tested in the manner described. I would like to ask Dr. Stetten whether he has

done experiments of this type.

DR. STETTEN: We expect, Dr. Stadie, that our material, which is an evanescent material, may be different from the material which you observed or which Haugaard and Marsh observed in this particular regard. We have only one experiment relating to this point, but in one experiment with this remarkably resistant serum, diaphragm was immersed in the serum, taken out, rinsed, and then treated with insulin, and still no insulin effect was observed. On this ground it is suggested that the material which makes for the insulin resistance in ketosis, in acidosis, may be different from that which Haugaard and Marsh observed in the more chronic insulin resistance. I believe all their patients were insulin resistant for prolonged periods of time.

DR. KRAHL: I have been very much interested in Dr. Stetten's remarks about this inhibitor. Two or three years ago Dr. Bornstein, Dr. Park, and I found in the plasma of severely diabetic rats an inhibitor which would reduce the glucose uptake of diaphragm. This inhibitor was in one fraction of the diabetic plasma, namely, the lipoprotein. We have continued to be interested in this; I wonder whether Dr. Stetten has any information about whether his inhibitor is in this fraction or not.

DR. STETTEN: May I ask one question? This material was unstable at low temperature?

DR. KRAHL: Freezing.

DR. STETTEN: Unstable?

DR. KRAHL: Yes.

DR. STETTEN: Ours is stable at freezing, and on this ground we have been inclined to think that it is at least different in this physical property.

CHARLES H. BEST, M.D., (*Toronto*): I am sure you will all agree this has been a particularly interesting morning session and that we should congratulate those who organized it, and the speakers.

It seemed to me that the tone of this meeting was set extremely well in cautious and very careful looks at some of the methods that are used in trying to determine how insulin really acts, and that the speakers seemed also to criticize in a fair and constructive way some of the previous work—including certain of their own findings. I found the meeting extremely stimulating, largely, I think, because of the emphasis on solid fundamental facts.

We are all very happy when any of our own experiments are confirmed. I have had occasion to look over the notebooks which Banting and I kept in 1921, and in 75 instances in 10 diabetic dogs we recorded a very definite fall in blood sugar with our pancreatic extracts. There were no exceptions. Therefore, we were not surprised when people in all parts of the world confirmed the fact that insulin lowers the blood sugar under most conditions!

The mechanism of action of insulin has fascinated me over all these years and is still left in a very unsettled condition. I always feel that one should look very carefully at findings which are apparently out of line in the hope of gaining new leads. The toxicity of growth hormones in hypophysectomized animals is one of these. Dr. de Bodo's work has been done with great care and undoubtedly is correct. But this is an extraordinary finding. We should continue as he has done to look at different preparations to see if this is a real physiological effect or perhaps connected with something that is not physiological.

Diabetogenic Action of the Pituitary

Clinical Observations

E. Perry McCullagh, M.D., Cleveland*

All thoughtful students of diabetes are well aware of the fact that carbohydrate metabolism is controlled by the interplay of a variety of factors. The average clinician gradually is coming to realize the importance of studies of these factors, for with a better understanding of human diabetes there will come better control and eventually, it may be hoped, prevention or permanent cure of the disease.

The known and theoretical causes of human diabetes continue to multiply. In addition to failure of insulin production, we think of the possibility of disturbances such as excess of glucagon or excess of immune-like bodies or other blocking agents affecting insulin efficiency.

It appears likely that hyperglycemia if sufficiently great or of sufficient duration may precipitate lasting diabetes in some patients. In them, no matter what mechanisms are involved etiologically, it also seems likely that eventually true insulin deficiency may supervene. However, there is evidence that such diabetes may be both severe and temporary as illustrated by the remarkable case reported by del Greco and Scapellato.[1] In their patient the intake of huge quantities of sugar and honey was followed by hyperglycemia, severe glycosuria, and well-authenticated typical diabetic coma. The patient later completely recovered not only from the coma but from the diabetes as well, the glucose tolerance becoming *normal*. The reversal of diabetes or its disappearance is an unusual occurrence, but each piece of evidence produces renewed hope for eventual means of a cure.

The study of diabetic conditions that are associated with the pituitary or the adrenals therefore is of special value not only in revealing complexities of carbohydrate metabolism but also in showing us methods by which we gain a little more insight into means of reversing diabetes.

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With regard to the adrenal cortex, there are three types of diabetes seen clinically. The first is that which begins with pituitary hyperactivity such as in cases of pituitary tumor, excess adrenal stimulation, and Cushing's disease. The second is that seen in Cushing's syndrome or after excess exogenous cortisone. It appears to be identical with the first type except that it originates in the adrenal itself. In most of these, the diabetes is reversible. The third type which up to the present is rare is that of an aldosterone-producing tumor. I have observed three patients having proven aldosterone-producing tumors. Apparently in these patients diabetes is reversible, but there is no clear evidence of control of aldosterone production by pituitary function.

As to the adrenal medulla, pheochromocytoma is not infrequently associated with diabetes which is almost always cured so far as one can judge by glucose tolerance curves after removal of the tumor. The excess production of adrenalin or noradrenalin seems to bear no relation to pituitary function.

Such a preamble is all by way of saying that pituitary hormones and hormones related to pituitary and adrenal function are only parts of a total complex having intimate connection with hyperglycemia and with some instances of disease which we at present classify as diabetes mellitus. The pituitary hormones which we think of as intimately connected with the cause and persistence of some cases of human diabetes are:

1. growth hormone, as in acromegaly;
2. excess ACTH, as in the rare instances of pituitary tumor with Cushing's disease; and possibly
3. another factor which we might call "the Cori factor" bearing on hexokinase activity and which may be neither ACTH nor growth hormone.

ANTERIOR PITUITARY DEFICIENCY AND DIABETES

It is now 32 years since Olmsted and Logan[2] called attention to the fact that pituitary activity had a striking effect on animal sensitivity to insulin, and all of you are familiar with the early classical experiments of Houssay.[3] In the human being, severe anterior pituitary failure superimposed on diabetes is a rare occurrence.

Perhaps a dozen outstanding examples are to be found in the literature. Four such patients with an added one of his own were cited by Feldman and associates[4] in 1947. More recently reported instances are those by Marzullo and Handelsman,[5] by Poulsen,[6] and by Williams.[7] It is remarkable that although the general effects of Simmonds' disease were severe in all of these, the effects on the pre-existing diabetes differed markedly. In Calder's[8] patient a glucose tolerance test previously diabetic in type became normal, and in Vandenbroeck and Ferin's[9] patient the insulin dose fell from 60 to 43 units per day. On the other extreme, Kotte and Vonderahe's[10] patient who had acute pituitary necrosis, previously had had "excessive glycosuria" which disappeared within two days after pituitary destruction. The blood sugar levels in that patient fell to 31 and finally to 20 mg. per 100 ml. Feldman's[4] patient had taken 35 units of insulin daily but as pituitary cachexia developed this dosage was discontinued; the woman subsequently died in hypoglycemic crisis. The course in Williams'[7] patient who had required 60 units of insulin per day was similar. After pituitary necrosis, hypoglycemic crisis developed which resulted in her death despite the intravenous administration of 60 gm. of glucose.

There are other instances more recently reported, some of which will be mentioned in relationship to acromegaly. In 1953 Alexander[11] reported the case of a 36-year-old man who had had diabetes since the age of 17 years. He had had diabetic coma but for the most part had managed well on approximately 65 units of insulin daily for many years. Although there had been no obvious injury or intercurrent illness, he was found at 1:30 a.m. in convulsions from which he recovered after being treated by the oral administration of sugar. The next night at 11:00 p.m. he became comatose; at 2:00 a.m. he had convulsions in spite of 55 gm. of sugar intravenously. The blood sugar level fell to 8 mg. per 100 ml., and although it rose within the next two hours to 148 and the following day to as high as 400 with ketonuria, nevertheless consciousness was never regained and he died three days after his first very severe convulsion. At autopsy he was found to have anterior pituitary necrosis with some degenerative changes in the pons. Liver glycogen was found to be increased as was to be expected.

Brenner[12] recently reported a somewhat similar instance of a woman who had had diabetes for seven years and had been taking 10 units of regular or NPH insulin daily. She was admitted to the hospital because of septicemia which was thought to be due to *Escherichia coli*. She had been passing between 14 and 25 gm. of sugar in her urine per day. On the sixth hospital day she became lethargic and incontinent. Insulin administration was discontinued and the glycosuria disappeared. On the second and third day following the cessation of insulin, fasting blood sugar levels were found to be 11 and 28 mg. per 100 ml., despite the fact that on the second day following the cessation of insulin she was given 325 gm. of sugar intravenously. The patient died during the afternoon of the third day following cessation of insulin. Autopsy revealed extensive pituitary necrosis although there were some areas containing pituitary cells that appeared to be intact.

It is remarkable that, in instances of severe pituitary deficiency, sensitivity to insulin is very much greater than that seen in Addison's disease. This extreme sensitivity is dramatically illustrated by the report by Herstmann[13] of a patient who had severe hypopituitarism. During investigation she was given a test dose of 8 units of insulin intravenously. She developed hypoglycemic coma and died despite the use of intravenous glucose. Patients with hypopituitarism may react powerfully to as little as one unit of intravenous insulin.

A great deal of interest has centered about the effect of hypophysectomy on diabetes mellitus since Poulsen[6] observed disappearance of all diabetic retinopathy after the occurrence of Simmonds' disease in a woman 37 years of age. The eye signs consisted of punctate hemorrhages as well as larger retinal hemorrhages appearing as spots or stripes. There were a few remaining evidences of retinopathy four years after the development of pituitary cachexia. Seven years after its development, however, all evidences of this complication seemed to be gone. Since that time hypophysectomy has been performed in some cases of human diabetes in an attempt to halt the progress or to improve degenerative disorders, particularly retinopathy. To date the results have not been very encouraging.

HYPOPHYSECTOMY

Kinsell and associates[14, 15] have reported the results of hypophysectomies in four patients. The ages of the patients were respectively 31, 26, 30 years, and unstated. All patients had severe vascular complications and renal insufficiency. One patient died of renal and cardiac failure five months postoperatively; another died of coronary occlusion three months postoperatively. Two were alive at the time of the first report. The patients developed the expected extreme insulin sensitivity which was balanced to some extent by doses of cortisone. There was a lowering of urinary albumin and a diminution in hypertension, but no certain improvement in retinopathy. The most recent report[15] presents the results in six diabetic patients who underwent hypophysectomy.

They had been followed for as long as 18 months. Each had had a large decrease in insulin requirements. The average dose of insulin on a relatively high carbohydrate intake was less than 15 units per day. Hypertension, if present before hypophysectomy, disappeared postoperatively. However, there was still no conclusive evidence of improvement in the degree of retinopathy.

Luft and associates[16] reported the early results of hypophysectomy in seven patients having severe vascular degeneration and diabetes. Three of those patients were alive one, two, and one-half years respectively, postoperatively. One of them, a man, is living the ordinary life of a blind man. He is married and has normal sexual power. The fact that his daily insulin requirements dropped from 60 to 8 units suggests considerable pituitary deficiency. The fact that his sexual potence remains is to my mind, despite tests to the contrary, highly suggestive that a total hypophysectomy was not accomplished. Another patient is living but was not discharged from the hospital for 13 months after the operation. She had no hypertension preoperatively. Her insulin requirements fell markedly. There was no measurable improvement in the extreme retinopathy and no definite improvement in renal function. The third living patient developed severe hypoglycemia which was extremely difficult to manage and was in the hospital nine months postoperatively. Subsequently, vision was said to be no worse than it had been before operation. Of the other patients, one did not recover consciousness postoperatively. A second patient died nine days after surgery with severe hypoglycemia, epileptic convulsions, and fever which had recurred whenever cortisone was discontinued. The third patient who died developed generalized convulsions immediately after operation. She had the typical marked fall in insulin requirements; the blood pressure fell from 150/105 to normal. Subsequently, her blood pressure rose somewhat; and 15 months postoperatively she became comatose, had jacksonian epilepsy, and died. The autopsy showed cerebral hemorrhage. The fourth patient who died had severe hemorrhage at the time of surgery, many bouts of generalized convulsion postoperatively, subsequently a temporary recovery, and then gradual deterioration of his condition culminating in death five months postoperatively (figure 1).

Recently, Luft and associates[17] reported a total of twenty patients with diabetes who had been hypophysectomized and followed for 3 to 43 months. Death occurred

INSULIN REQUIREMENTS BEFORE AND AFTER HYPOPHYSECTOMY
(FROM LUFT ET AL.)

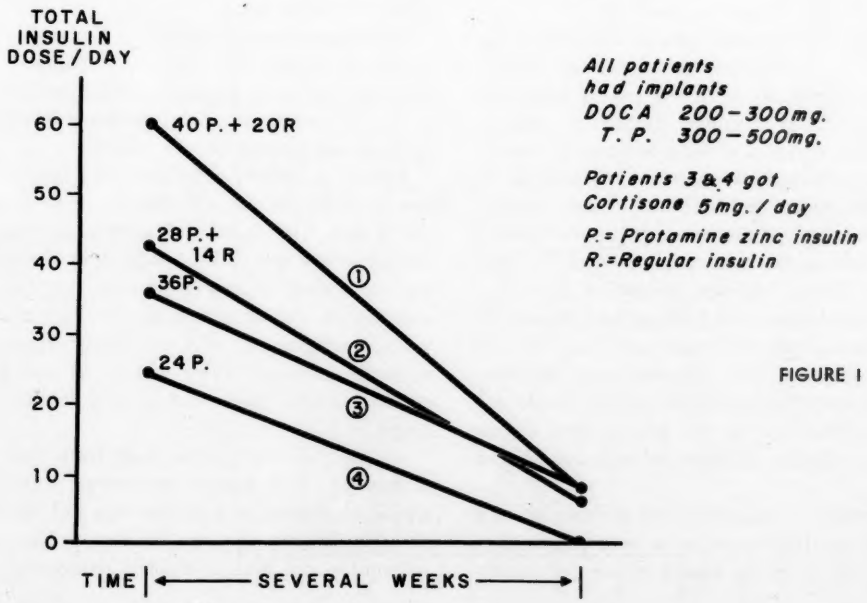


FIGURE 1

in 7 of the 20 between the first day and 19 months after operation. In the thirteen surviving patients there was a fall in blood pressure, a decrease in heart volume, and a decrease in albuminuria. In 7 of the 13 patients the visual acuity was said to be improved.

It would appear, in general, that much conservatism is still required in considering the advisability of creating such a severe disease as hypopituitarism in an attempt to halt the progress of progressive vascular disease.

Recently we have attempted to produce hypopituitarism in diabetes associated with severe retinopathy by means of doses of irradiation exceeding 10,000 r to the pituitary. Cobalt teletherapy was used. To the present we have failed to produce measurable pituitary deficiency. Except in acromegaly there has been no significant fall in insulin requirements, blood sugar levels, or urinary steroid titers (figures 2 and 3).

HYPERGLYCEMIA FOLLOWING THE ADMINISTRATION OF ACTH

With regard to the effects of ACTH in man, Cohn and Kolinsky[18] have shown that temporary diabetes mellitus may appear following the use of relatively large doses of corticotrophin.

In 1953 Zimmerman, Parrish and Alpert[19] reported the effects of injections of 40 to 100 mg. of ACTH daily for 5 to 12 days in 17 patients with various conditions, only one of whom had diabetes mellitus. The responsiveness to insulin was measured by the glucose-insulin tolerance test. Interestingly enough, in this group there were only four patients in whom there was a significant loss of insulin sensitivity.

Recently Martin and Pond[20] cited a very interesting instance of pituitary insufficiency in diabetes and showed that here, where apparently ACTH was lacking as judged by extremely low levels of urinary steroids, the administration of ACTH caused a striking increase in insulin requirement and a considerable increase in glycosuria.

As has been stated cortisone may cause marked aggravation of diabetes if present in Addison's disease; it probably would do the same to some degree in Simmonds' disease. In an occasional susceptible individual the diabetes is precipitated, or unrecognized diabetes is aggravated. For example, in Fajans and Conn's[21] case which was being treated with ACTH because of leukemia there was great intensification of the diabetes. There was glycosuria to as much as 100 gm. per day over dietary intake, 23 gm. negative nitrogen balance, and insulin resistance.

It can be confidently anticipated that diabetes existing in latent form may remain severe in some people after it has been made severe by steroid effects. Fortunately such must be the case very seldom.

Bookman and associates[22] cite five patients who developed diabetes during cortisone or ACTH therapy. It disappeared in four. The other patient probably had diabetes before treatment as there was glycosuria ten years previously. That person was given cortisone for a period of two years, usually 75 mg. daily, and required 10 to 20 units of insulin per day.

Lasting diabetes may actually have been caused by ACTH in Bishop and Glyn's case.[23]

SPONTANEOUS STEROID DIABETES

Steroid diabetes arising spontaneously is due to 11-oxysteriod-like materials. They cause glycosuria partly by a direct effect on the renal tubules and also by interfering with the action of insulin, and they cause glycosuria and hyperglycemia by impairing carbohydrate utilization and by augmenting the breakdown of protein. Clinically such diabetes is relatively mild, relatively insulin insensitive, and associated with a negative nitrogen balance which is not fully corrected by insulin. It is usually temporary, disappearing with the correction of the steroid excess upon which it depends.

It is a remarkable fact that Cushing's syndrome of long standing and of severe grade may be present without diabetes. It would seem evident that important protective homeostatic mechanisms come into play. In some cases steroid diabetes is associated with hypokalemic hypochloremic alkalosis. This was true in the case reported by Sprague, Hayles and others,[24] in which relatively large amounts of 17-hydroxycorticosterone were isolated from the urine.

Syndromes showing a mixture of Cushing's and adrenogenital syndrome may also show diabetes; we have seen one instance in which the clinical picture appeared to be purely an adrenogenital syndrome in which severe alkalosis was present but no diabetes.

Among 34 patients with Cushing's syndrome, we have seen 21 with diabetes. Of these there were 15 women and 6 men. The number of patients in whom insulin was prescribed was 7. The lowest dose of insulin was 8 and the highest dose was 90 units per day. Vascular complication usually associated with hypertension is almost an integral part of Cushing's syndrome, and consequently it is not always easy to be sure how much of the vascular disease can be said to be of diabetic origin.

Among our own patients there were three who had retinopathy. One was a hemorrhagic retinopathy not typical of diabetes in a patient who had blood pressure of approximately 175/120. In two patients there was retinopathy classified as diabetic retinopathy. One had

157 357

BLOOD SUGAR AND INSULIN DOSE AFTER COBALT TELETHERAPY 12,000 r. TO THE PITUITARY IN DIABETES MELLITUS

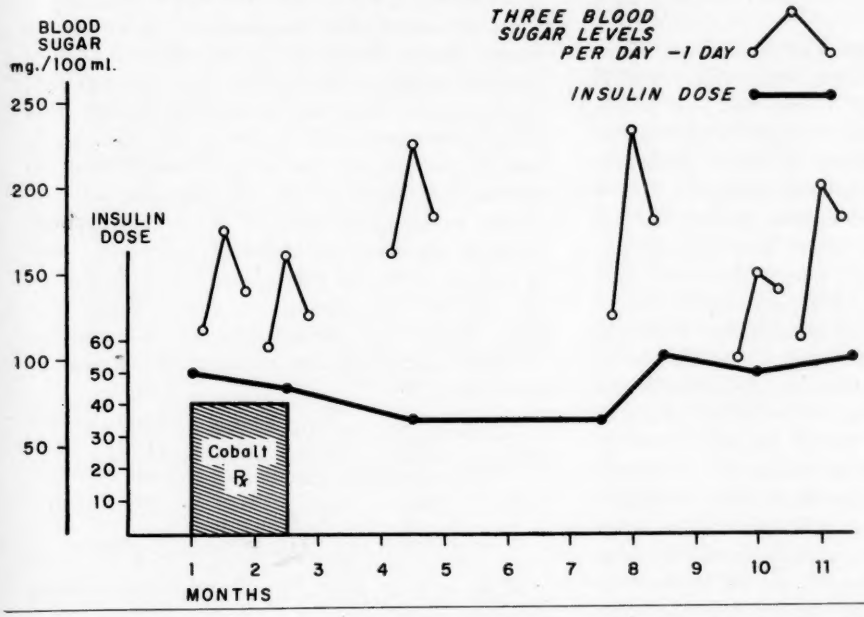


FIGURE 2

157 357

COBALT TELETHERAPY 12,000 r. TO THE PITUITARY IN DIABETES MELLITUS

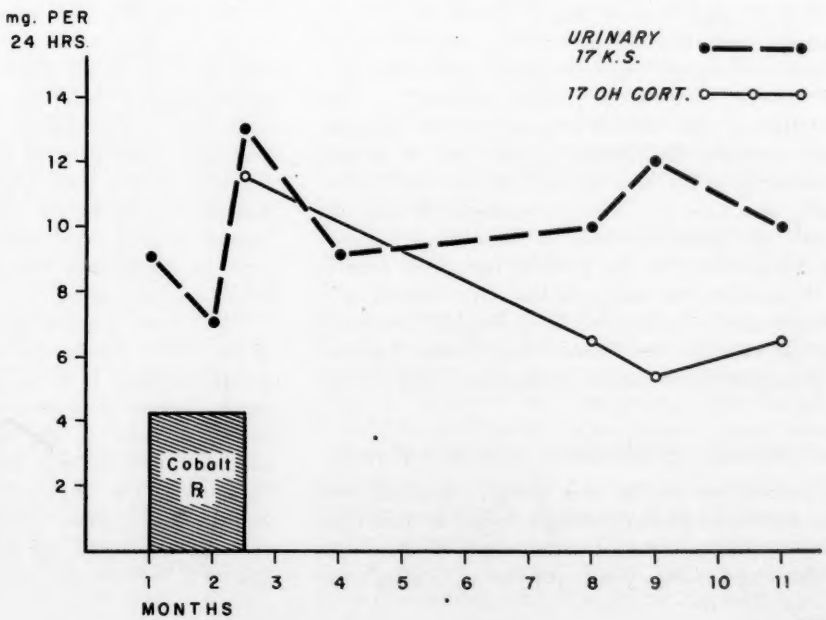


FIGURE 3

features of Cushing's syndrome for 11 years and diabetes for 9 years before the retinopathy was found. In the other patient the blood pressure had been as high as 196/128. The diagnosis of Cushing's syndrome and diabetes was made in 1936 and the retinopathy eight years later.

The possibility that certain chemical features of the diabetes in Cushing's syndrome differ from those in the average case of clinical diabetes had been studied by Hills, Power, and Wilder.[25] They showed that in 34 patients with mild diabetes the blood lactate and pyruvate levels were also equivocal. Among six patients with Cushing's syndrome and diabetes mellitus, four had pyruvate and lactate levels which were markedly elevated before glucose was given for a glucose tolerance test and even more elevated after the administration of glucose, differing in this way from patients with mild diabetes without Cushing's syndrome. Among the six patients with Cushing's syndrome and diabetes who were studied, two had hypokalemic hypochloremic alkalosis.

It is impressive to see that in steroid diabetes of long standing there may be no tendency to permanence of the diabetic state. For example, one of our patients had glucose tolerance curves that were diabetic before surgery. When postoperative adrenal insufficiency arose the diabetes not only disappeared but the tolerance became that typical of Addison's disease. It is interesting, however, that two of our patients who had severe Cushing's syndrome and who had complete clinical recovery following adrenal surgery both suffered severe myocardial infarction ten years following the surgery.

It is of special interest that it has not been shown that the steroid diabetes of Cushing's syndrome causes diabetic retinopathy more frequently than other types of diabetes. This may be partially attributable to the fact that in most patients with the diabetes of Cushing's syndrome the diabetes had not been of as long duration as it has been in most patients with retinopathy who have no Cushing's syndrome. Because the "cure" of Cushing's syndrome is almost always followed by disappearance of the diabetes, the author believes it is unlikely that adrenocortical hyperfunction is a common cause of diabetes mellitus, especially since those patients who have been known to have adrenal deficiency superimposed upon diabetes mellitus have persistent diabetes.

CUSHING'S SYNDROME AND CUSHING'S DISEASE

Probably no one has had enough experience with the diabetes of proven Cushing's disease to draw absolute conclusions about it. Since the identity of the diabetes of Cushing's disease and that of Cushing's syndrome is to some extent an assumption, it will be dealt with briefly. Almost all I can say refers to diabetes associated with adrenal hyperplasia or tumor without evidence of pituitary tumor or hyperactivity.

In Cushing's syndrome associated with adrenal cortical hyperplasia, diabetes may disappear after subtotal adrenalectomy. Such a reversal of the hyperglycemia is illustrated here by repeated glucose tolerance curves (figure 4). Instances of this type in which the disease emanates from pituitary tumor are usually classified as Cushing's disease; however, they are rare. In one such patient whom we observed, the diabetes, which was very mild, was presumably caused by an excess of ACTH. It disappeared following the local application of radioactive cobalt on a thread which was inserted into the pituitary gland and later withdrawn through the wound. There was a subsequent recurrence, and death followed adrenalectomy.

Glucose tolerance before treatment of the pituitary tumor was:

Fasting	½ hour	1 hour	2 hours	3 hours	4 hours
136	240	370	275	116	

and six months later it was:

137	208	149	110	75	86

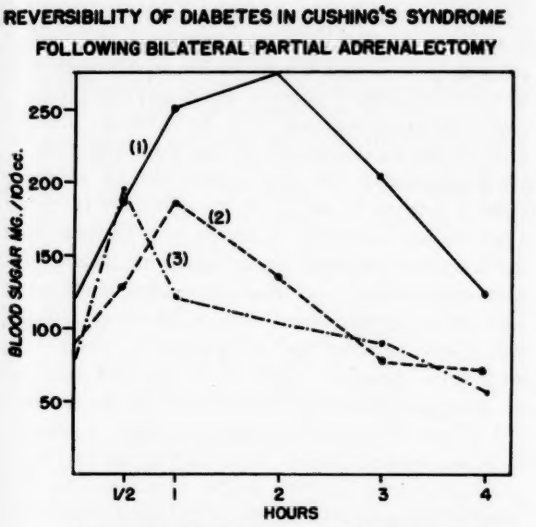
THE PITUITARY DIABETOGENIC FACTOR

So far as I am aware, there is no clinical evidence that there is an excess of a pituitary hormone which interferes with hexokinase activity and which is separate from ACTH or growth hormone.

ACROMEGALY AND DIABETES

The effect of growth hormone upon human diabetes has been studied in one patient by Kinsell, Balch and Michaels.[26] A "highly purified" growth hormone preparation was used. Under controlled conditions this caused an increase in hyperglycemia and glycosuria, and there were minimal evidences of ketonuria. The nitrogen balance changes also were minimal. The authors thought these findings favored the idea that the effect of growth hormone resulted more from interference with the utilization of insulin than from gluconeogenesis caused by breakdown of proteins.

The diabetes of acromegaly is the clinical counterpart of the type of diabetes that is caused experimentally by growth hormone. It is not possible to state with certainty, however, in a given case whether an excess of growth hormone is the sole responsible factor, or whether that factor plus some excess of ACTH, with incident excess of oxysteroids, may be involved. It is true that large adrenal cortices have been found commonly in acromegaly, and the findings of Venning and Browne[27] indicate that increased excretion of corti-



(1). Preoperative. (2). Two months postop. (3). 1½ years postop.
Body weight at time of first test, 122 lbs.; at time of last test, 108 lbs.
Glycosuria 1.23 gm. on preop. tests; none on postop. tests.

FIGURE 4

costeroids may occur. If in acromegaly excess ACTH is produced one would expect the frequent development of Cushing's syndrome in acromegaly, but they rarely coexist. One such case is that reported by McCormick and others.[28] In their patient there was apparently an excess of ACTH according to assays done by Dr. A. Albert.

Most critical observers are not satisfied with the reliability of methods currently available for the estimation of excesses of 11-oxysteroids, and those methods most recently developed have not yet been fully evaluated in acromegaly. It is to be hoped that such new methods as that of Sydnor and Sayers[29] for blood ACTH will help to solve this part of the problem.

In our laboratory we are currently utilizing a method for measurement of growth hormone in which amounts of purified pituitary extracts as little as 5 micrograms can be measured. In our hands and in others clinical growth hormone studies have not yet shed any new light on diabetes mellitus.

Some interesting studies along this line have recently been reported by Randle[30] working with Professor Frank Young at Cambridge, England. He has measured plasma-insulin activity in acromegaly. He points out a very interesting fact with regard to assay for insulin. He believes that, if the alloxan-diabetic-hypophysectomized-adrenalectomized rat is used, the investigator is actually measuring a balance of effects of insulin versus insulin antagonists. If, for example, an excess of glucagon or some substances which cause a relative insulin immunity might be present, the measured effect would indicate the presence of less insulin than was actually in the solutions tested. Randle believes that, if the glucose uptake of the rat diaphragm is used as a test object, glucagon, if present, will not be measured.

Using this method, he measured insulin-like activity in normal and diabetic patients and in acromegaly. He showed that a diabetic patient receiving insulin had more measurable insulin in the plasma than a normal person. Plasma from acromegalics studied showed a great deal of insulin activity; in fact, measurably more than normal in plasma diluted up to 100 times. He substantiated his findings by showing that he was capable of measuring as little as 2 milliunits of insulin as such; moreover, he showed that he could measure an increased insulin effect in normal plasma diluted four times and collected two and one-half hours after oral glucose. At this point the plasma contained not only more insulin than it did in the fasting state but about as much as was measured as 2 milliunits.

Daughaday, Perry, and MacBryde[31] showed an increase in urinary "cortin" by the Formaldehydogenic method in their insulin-resistant case.

The incidence of diabetes in acromegaly has been variously estimated. The most frequently quoted figure is that of Coggeshall and Root[32] who found that of 153 acromegalics 35 per cent had glycosuria and 17 per cent had diabetes. We think it is fair to assume that many more than 17 per cent could be classed as diabetics if glucose tolerance tests had been done in all of them. It may be pertinent, however, that ACTH and cortisone cause glycosuria before hyperglycemia occurs as a result of their action.[31] With the experimental facts in mind it is not easy to understand why so few acromegalics have diabetes.

The time relationship between the beginning evidence of acromegaly and diabetes varies tremendously according to available figures. Coggeshall and Root's analysis shows a variability from 1 to 22 years, and the figures of Goldberg and Lisser[33] are similar. In our patients diabetes and acromegaly were present together when the diagnosis was first made in one, and the acromegaly was known to be present for 15 years before diabetes was found in another; in the remainder the time interval between the beginning of acromegaly and of diabetes apparently lay between these extremes.

In 76 acromegalics in our series, there were 21 classified as diabetic. It seems evident that mild diabetes, which is disclosed only if a glucose tolerance test is

done, is present in many acromegalics and for an unknown length of time before it is demonstrated.

Diabetes is said to occur much more commonly in women with acromegaly than is true in women generally. In our series there were 9 women and 12 men. In Coggeshall and Root's series the diabetes was also much more common in those acromegalics who had a diabetic family history.

While most acromegalic diabetes is mild, severe diabetes does occur. Three of Coggeshall and Root's 16 fatal cases, for example, died in diabetic coma. Insulin resistance occurs, and it is a common impression that it occurs more frequently in acromegalics than in the average diabetic. We agree with this impression, but we cannot support it with accurate statistics. We have one patient, an acromegalic man of 48 years, who requires 160 units of insulin per day for maintenance. There are two others who have required 220 and 175 units daily for brief intervals but usually require doses of about 60 and 80 units respectively.

The muscular wasting and the signs simulating amyotrophic lateral sclerosis do not parallel the severity of diabetes in acromegaly. The muscular weakness may be extreme, or actual amyotrophic lateral sclerosis may be present, with very mild or no diabetes; or diabetes may be severe with no evident muscular wasting or weakness.

Arterial hypertension, some arteriosclerosis, and intracranial vascular accidents are common in acromegaly. Diabetic retinopathy has been reported in acromegaly.[32] We have seen typical diabetic retinopathy in 3 of 21 acromegalic diabetics. One was a man who had had acromegaly for 12 years and diabetes for 8 years when retinal changes were first seen. His blood pressure was 160/100. In another a few punctate hemorrhages were found in a man who was known to have had diabetes only two years. His blood pressure was 110/60. The third patient was a 28-year-old man who was found to have retinal hemorrhages which were actively recurring. He had been known to have acromegaly for 10 years. He had had improvement in visual fields and apparent slowing of acral growth after operation upon a pituitary tumor in 1944. Despite this he developed diabetes two years later which was five years before retinopathy was found. His blood pressure was 120/90. Neither coma nor gangrene has been seen in any of our patients. The type of renal failure seen in chronic diabetes, if present in our acromegalics, has not been recognized by us. The paucity of such vascular complications may be related to the fact that most acromegalics die before their disease has lasted fifteen years, which means the diabetes has usually existed for a much shorter time.

It is mentioned frequently in the literature that there is an unusual variability in the severity of diabetes of acromegaly. In a sense this is true, but in our experience after watching the progress of acromegaly for years we have been more impressed with the fact that in most such patients the diabetes is mild and peculiarly stable—vastly different in this respect from that of so many diabetic patients in whom we struggle daily to try to attain diabetic control. It is equally true, however, that diabetes may show ingravescence during maintained pituitary hyperfunction and slow or sudden amelioration with decline in hypophyseal function. However, Darragh and Shaw[34] reported the development of diabetes in acromegalic patients in spite of the apparent arrest of the acromegaly following X-ray treatment of the pituitary.

The case of Almy and Shorr[35] is most instructive. A 40-year-old acromegalic with diabetes for five years developed mastoiditis and basilar meningitis. He had required 60 units of insulin per day. Later in association with evidence of pituitary failure his glucose tolerance became normal and remained so for five years. In John's[36] case there was spontaneous disappearance of diabetes in a patient with acromegaly.

Balfour and Sprague[37] had a somewhat similar experience. Their acromegalic patient, who had had blood sugar levels as high as 384 mg. per 100 ml. and who required 62 units of insulin daily, following X-ray therapy had an acute episode thought to be due to rupture of an intrasellar tumor. Later 10 units of insulin daily controlled the blood sugar to a level at 147 mg. per 100 ml.

Recently, Gurling[38] has reported from London an instance of an acromegalic man 49 years old who had diabetes with blood sugar as high as 740 mg. per 100 ml. and mild acidosis with severe coma and fever. The hyperglycemia was controlled with therapy. Temperature continued to rise, and coma continued and led to death. Hypoglycemia in this instance apparently did not develop, although death was associated with pituitary necrosis.

In our own experience[39] reversal of diabetes in acromegaly has been seen to follow long-continued therapy with estrogen. A report of this experience has recently been published. This report concerned six acromegalic women in five of whom there was diabetes mellitus. In all of these the diabetes was mild as shown by glucose tolerance tests. The doses of estrogen used were very large. Stilbesterol was given in doses of 10 mg. to as much as 50 mg. per day, or ethinyl estradiol 1.0 to 5.0 mg. per day, which is 20 to 100 times the usual dose. The changes were slow, taking months to occur. Although the patients were not on carefully regulated

diets, we believe the changes are significant because they were progressive; they were consistent in all five patients; and in addition, after the glucose tolerance became normal or nearly normal during therapy, cessation of treatment was followed in three cases by a recurrence of the abnormal glucose tolerance test. Growth hormone production was apparently suppressed because there was soft-tissue shrinkage and a sustained lowering of blood phosphorus levels.

The change in glucose tolerance we believe was due in part at least to diminished growth hormone production. An example is shown in figure 5.

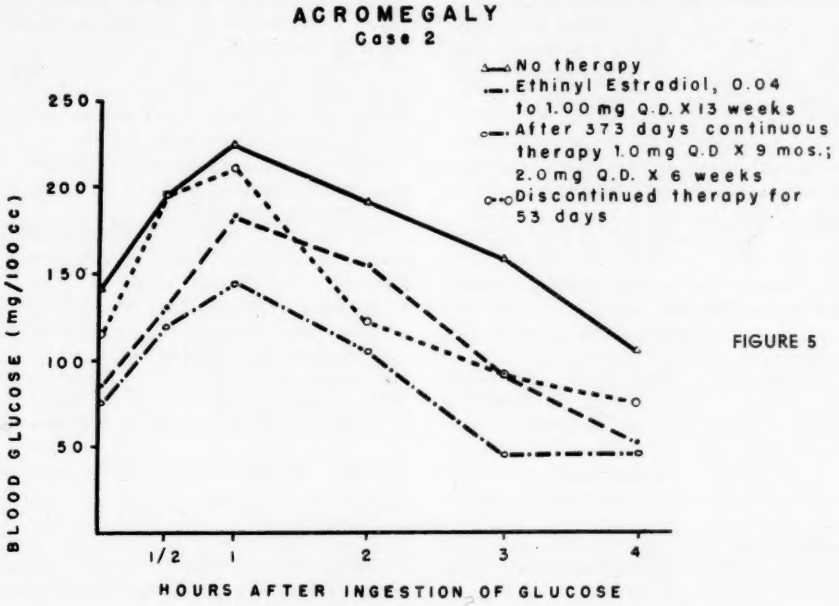


FIGURE 5

SUMMARIO IN INTERLINGUA

Action Diabetogene del Glandula Pituitari: Observationes Clinic

Diabete pote esser associate con hyperpituitarismo (in acromegalia, morbo de Cushing, etc.), con hyperadrenocorticismo, e a vices con pheochromocytoma. Le involvite hormones pituitari es hormon de crescentia e ACTH.

Deficientia antero-pituitari in humanos produce un typic phenomeno de Houssay. Forte doses de radios X o teletherapia a cobalt in doses de usque 12.000 r non ha succedite a producer hypopituitarismo. In certe casos, disfallimento pituitari seque hyperpituitarismo, es associate con extreme sensibilitate a insulina e a vices morte hypoglycemic.

Ben que hypopituitarismo ha essite sequite in certe casos per melioration del retinopathia diabetic, effortios a producer tal resultatos per medio de hypophysectomia ha usque nunc essite associate con multiple complicationes.

Le administration de ACTH pote esser sequite per diabete, sed diabete perdurative post ille tractamento es quasi incognoscite. Le diabete del syndrome de Cushing es usualmente leve, relativemente sensibile a insulina, e, como regula general, completemente reversibile post le effectuation chirurgic de controlo del syndrome de Cushing.

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DISCUSSION

OLOF H. PEARSON, M.D., (*New York*): I have very little to add to the very able presentation by Dr. McCullagh on clinical observations of the relationship of the pituitary to diabetes. I thought it might be of some interest, however, to mention a few meager observations which we have on carbohydrate metabolism in patients who have undergone total hypophysectomy. These are patients with cancer. None had diabetes.

Following total hypophysectomy, with the patients maintained on cortisone and thyroid in what we considered to be physiologic maintenance dosage, we have

noted that the fasting blood sugar tends to be about 10 to 20 mg. lower than their preoperative fasting blood sugar. Their insulin tolerance is somewhat decreased. About one third of the usual dose of insulin will produce a normal insulin tolerance curve. They have a normal hypoglycemia responsiveness. Thus, the changes in carbohydrate metabolism in the totally hypophysectomized patient are relatively small.

We have seen no instances of symptomatic hypoglycemia in any of about 100 patients we have studied.

In the patients maintained only on cortisone without thyroid—these patients are hypothyroid but not severely myxedematous—we have seen no obvious differences in carbohydrate metabolism as measured by these parameters. If we withdraw cortisone from the hypophysectomized patients, the majority of these patients will develop adrenal crisis within a period of a few days. Under these circumstances, hypoglycemia also is not a very prominent feature. Even in patients who eat practically nothing for 24 to 48 hours, the blood sugar remains in the neighborhood of 50 or 60, and there are no symptoms that we can ascribe to hypoglycemic phenomena.

There are a few patients who have proven total hypophysectomy who can go for a few weeks without cortisone and thyroid therapy. They do not do well, but they are able to maintain life during this period. Again, we have seen no symptomatic hypoglycemia in these patients. We have not, however, tested insulin sensitivity or the effects of growth hormone under these particular circumstances. These cases, as I mentioned, were relatively few in number. Most of them will develop adrenal crisis very promptly on withdrawal of cortisone.

We have made observations on the effects of administering growth hormone in only two hypophysectomized patients. Both of these patients were maintained on cortisone. They were given beef pituitary growth hormone in doses of 100 to 200 mg. per day for a period of two to three weeks. We observed no diabetogenic effects whatever from this growth hormone preparation. These patients had normal glucose tolerance tests prior to administration of growth hormone, and their glucose tolerance remained exactly the same during growth hormone administration.

We did not test insulin sensitivity under these circumstances. We have no information as to whether growth hormone will restore the patient to a normal insulin sensitivity.

We have made one observation with the administration of prolactin in a totally hypophysectomized patient, administering 150 mg. a day for a period of three or more weeks. We observed no change in carbohydrate metabolism as measured by fasting blood sugars or glucose tolerance test.

We have had very little experience with the diabetes of acromegaly. However, there was one patient whom we were observing, contemplating the possibility of hypophysectomy, which turned out not to be necessary, because radiation seemed to produce a suppression of the activity of the disease. This patient had a relatively mild diabetes. Because of the peculiar effects of growth hormone in hypophysectomized dogs, which Dr. de Bodo mentioned, we thought it would be of interest to administer some cortisone.

Figure 6 illustrates our findings in this patient.

This patient, on 10 units of insulin, had fasting blood sugars of about 150 mg. per 100 ml. and was excreting about 5 gm. of glucose per day in the urine. Without insulin there was very little change. The fasting blood sugar did get up as high as 200 mg. When 100 mg. of cortisone acetate was administered by mouth, you will note that there was a prompt rise in glycosuria, reaching over 100 gm. of carbohydrate per day, with a moderate rise in the blood sugar. When the cortisone was withdrawn, there was a decrease in the glycosuria.

This would not suggest, at least under these circumstances, any antagonistic effect between cortisone and growth hormone. However, these are not the conditions, of course, in which Dr. de Bodo observed the very great sensitivity to growth hormone.

In conclusion, then, our observations at the moment indicate that total hypophysectomy in patients with cancer but without diabetes has relatively minor effects on carbohydrate metabolism, as evidenced by slight lowering of blood sugar and a slight increase in insulin sensitivity.

HERBERT POLLACK, M.D., (*New York*): In the middle 1930's, Dr. Rollin Woodyatt from Chicago reported the effect of X-radiation therapy on diabetes. One of these patients, I remember, he sent to me because she moved East, and we had the opportunity of following her until she died in 1948. According to Woodyatt's findings, she was apparently one of the very brittle diabetic patients, and after about 9,000 roentgens to her pituitary region her diabetes subsided into a perfectly average type of diabetes that was quite well controlled, at least with respect to glycosuria, with about 15 units of insulin a day. She maintained this improvement up until the time of her death.

On the basis of this experience, about 1937 we subjected a dozen patients to intensive X-radiation therapy to the pituitary region and followed them for a period of a year and a half in the clinic. Unfortunately, we

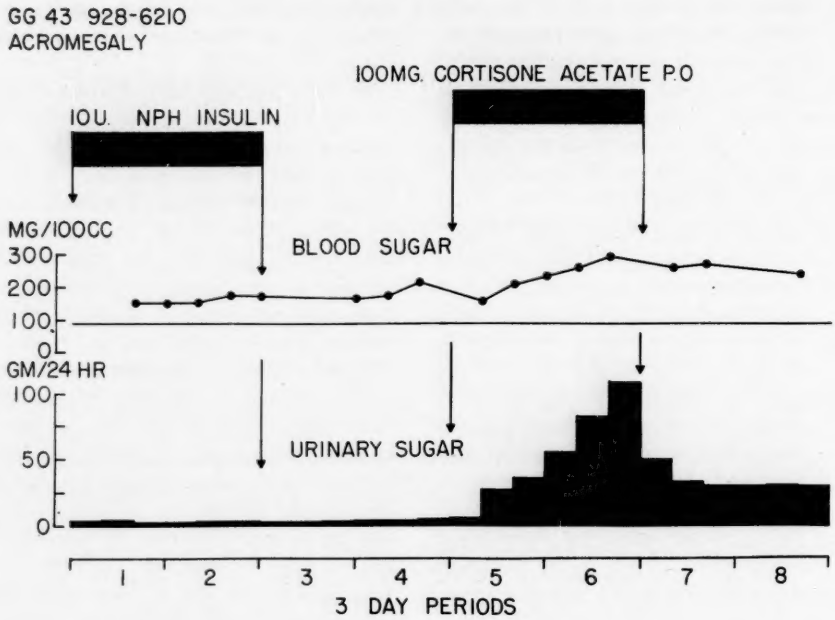

FIGURE 6

did not get the same results on this group, and we dropped radiation therapy.

But periodically this type of therapy does come up, and there is always just enough interest to keep us looking for the reasons why we failed.

DR. E. KORNFIELD: I would like to ask Dr. McCullagh whether he has had an opportunity to observe the effect of corticosteroids on patients with hyperinsulinism.

DR. MCCULLAGH: We have not used steroids for the treatment of hypoglycemia, except in those cases in which the hypoglycemia was thought to be of pituitary origin.

BENJAMIN KRAMER, M.D., (*New York*): Dr. Irvine McQuarrie has treated children suffering with hypoglycemia of unknown origin with ACTH and restored the blood sugar to normal. He was able to continue the effect over a long period of time and to reduce the dose to about 5 or 10 mg. every few days.

Hypoadrenal Function and Adrenalectomy in Human Diabetes

Irving Graef, M.D., New York*

The role of the adrenal gland in normal and abnormal metabolism is under constant study as reflected in the reports presented before this symposium and appearing elsewhere. No precise role in human diabetes has been defined. We know that the gluco-corticoids, epinephrine and norepinephrine all may affect carbohydrate metabolism through gluconeogenesis glycolytic action. In man the catechol-amines are not regarded as being significantly involved in the disturbances of diabetes mellitus. The possibility that the gluco-corticoids may be involved is still worthy of consideration. Their possible action through gluconeogenesis from protein and fat, or through interference with glucose utilization, their mobilization in homeostatic responses to stress, even to the metabolic stress of insulin-deprivation, insulin-lack, or their action as insulin-antagonists in diabetes need consideration. The absence of gluco-corticoids will at least prevent their contribution to the discharge or maintenance of surplus glucose however derived.

The suggestion has been made[1] that "insulin-sensitive" diabetes may be due to lack of endogenous insulin and that "insulin-insensitive" diabetes may be due in part to an excess of insulin antagonists. Crude anterior pituitary extracts, growth hormone, ACTH, lactotropin, as well as the 11-oxygenated steroids have all been shown to have "diabetogenic" properties and are suspected of playing roles in the insulin antagonizing mechanisms. Well-documented cases of "steroid diabetes" have been reported since the introduction of ACTH and the steroids in therapy.

In the absence of clinically apparent adrenal cortical disease no well-established evidence has yet been offered to implicate the steroids in ordinary human diabetes. In fact studies thus far reported have shown normal or deficient adrenocortical function in most patients with diabetes mellitus.[2, 3] However, bizarre patterns of urinary formaldehydogenic steroids, high or low, have been encountered in diabetic acidosis[4a, 4b] and unusually low values in pregnant diabetics[2] when compared with those in normal pregnancy.[5]

Izzo and Eilers[6] have recently reported a peculiar response to the oral administration of cortisone in a small group of patients with diabetes mellitus. Small and large doses of cortisone produced a significant rise in the excretion of 17-ketosteroids even though the control values were low normal or below the normal range as previously noted by Miller and Mason[7] and Lundbaek.[8] Izzo and Eilers observed a lesser rise in reducing corticosteroids in response to low and moderately high doses of cortisone which paralleled the rise in 17-ketosteroids. This fell to normal when the cortisone was discontinued. The meaning of this reported response to extra cortisone needs further study for elucidation.

EFFECT OF ADDISON'S DISEASE ON DIABETES MELLITUS

The reports of the coexistence of Addison's disease with diabetes mellitus, summarized by Markovitz[9] in 1954, provide a total of forty-three instances in which the destruction or atrophy of the adrenal glands did not prevent or modify significantly the clinical evidence of diabetes mellitus. Among the previously reported cases, he found that in five there was merely a statement of diagnosis without history or findings; in one there was no evidence of adrenal insufficiency in life. Four cases were reported prior to 1910 and are likewise incomplete; and two may have had hemochromatosis. In another, the patient had atypical Addison's disease. In the thirty remaining well-documented cases it was noted that in some, diabetes antedated the development of adrenal cortical insufficiency, in others it followed, and in a few, both developed simultaneously. Since his re-

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port, four more cases have been published including one in a ten-year-old boy.[10, 11, 12, 13]

It is generally suspected that the therapeutic use of crude adrenal extracts, before the isolation of pure hormones, probably induced the diabetic state in those patients with pre-existing Addison's disease of whom there are now six cases reported. The other types are a mystery in endocrine disharmony and challenge our present knowledge, but the diabetogenic action of the therapeutically employed steroids is obvious.

From the point of view of amelioration of diabetic nephropathy and retinopathy, it is regrettable that in none of the reports concerning the patients with pre-existing diabetes is it possible to determine that the supervention of Addison's disease—whether due to tuberculosis or so-called primary atrophy—led to anything more than a reduction of insulin requirement. There are no notes of the effect of the Addisonian state on pre-existing retinopathy, hypertension, or nephropathy attributable to vascular complications of diabetes. As might be suspected none had hypertension. Markovitz[9] noted that the sequence of events, diabetes first or Addison's disease first, did not materially alter the clinical or laboratory findings. As a result of these reports, it may be adduced that the development of a decreased insulin requirement in a diabetic patient warrants investigation. Whether or not the well-known decline of insulin requirement in the advanced Kimmelstiel-Wilson type of diabetes reflects a disturbance in the pituitary-adrenal functions remains to be studied.

EFFECTS OF ADRENAL AND PITUITARY DISORDERS

In considering the role of the pituitary and adrenal hormones, the experimental production of diabetes by pancreatic injury even in the adrenalectomized or hypophysectomized animal or in the combined adrenalectomized-hypophysectomized animal should be remembered.[14] Thus ablation of the pituitary and adrenal tissues need not prevent or check the course of such diabetes. Again one may say that their removal should at least reduce the contribution of the gluco-corticoids, especially in stress, to the surplus of circulating glucose however derived. That man and experimental animals can still obtain sufficient glucose for all energy purposes in the absence of gluco-corticoids has been observed in the survivors of the ablative operative procedures.

Are there any conditions in which diabetes may be worsened, brought to clinical notice, even initiated by hypercorticism? One thinks of Cushing's syndrome, pituitary neoplasms, adrenal cortical neoplasms, even tumors of the chromaffin tissue, but these are rare and the coincidence with the genetically-linked disease diabetes is almost impossible to establish. I have found no evidence of this sort in the literature. (Readers are referred to the paper of McCullagh[15] in this symposium for a fuller discussion of this aspect of the problem.)

RELATIONSHIP OF PREGNANCY TO DIABETES

Hoet[16] has suggested that diabetes following or developing in pregnancy may be linked to adrenal hypercorticism. This speculation has been partially supported by his and other studies.[17, 18, 19] If insulin antagonists are involved in injury to, or changes in, the fetal pancreatic islets,[20] then a case for a potent etiologic basis for a form of congenital diabetes may be linked to a kind of "hypercorticism" in the mother. If the placental hormones together with the adrenal gluco-corticoids and the pituitary-insulin antagonists prove capable of initiating or precipitating some forms of diabetes, then the question of the hormonal relationships should be further investigated both during and after pregnancy. Certainly the severe childhood and juvenile forms of diabetes appear during growth and active endocrine secretion.

Less can be said for the endocrine basis in explaining the appearance of diabetes late in life but even here the reciprocal hormonal relationships after the decline of the gonadal functions have not been examined fully in diabetes.

The argument may be made that severe progressive diabetes is possibly severe and progressive because of challenges to the pituitary and adrenal glands in everyday life with responses which burden a damaged or faulty carbohydrate regulating mechanism with surplus glucose, which normal individuals can store or utilize readily.

"MALIGNANT VASCULAR DIABETES" AND ADRENAL FUNCTION

Another aspect of the endocrine relationships concerns the peculiar kind of diabetes associated with widespread arteriolar-capillary damage. First properly recognized by Kimmelstiel and Wilson in the renal manifestations, it was earlier known to ophthalmologists as a hallmark of diabetes. Friedenwald,[21, 22] Becker[23] and McManus[24] linked the two by identification of the morphologic appearance of the arteriolar-capillary lesions in both sites, and by the constant presence of the retinopathic lesions in every instance of diabetic glomerulosclerosis. No less important is the widespread splanchnic arteriolar sclerosis or its presence in the peripheral arterioles. The association with hypertension and later renal insufficiency, the nephrotic picture, cardiac failure,

and coronary sclerosis are too well known to reiterate in detail. Friedenwald and Becker[25] have also claimed that the "Vitamin B_{12} test-dose excretion" provides a useful clinical test for the presence of retinopathy in the diabetic and they have linked the abnormal results to implied excessive or abnormal adrenal cortical function.

Kinsell[26] has termed this form "malignant vascular diabetes." As is well known, the diabetes may be mild or moderate. It may have been severe and often becomes mild—and usually becomes milder. All agree that when the triad of retinopathy, symptomatic renal glomerulosclerosis and diabetes is encountered together, the prognosis is grave. The younger the patient, the graver the prognosis. Even the best of "diabetic control" often has no influence on the course of this form of diabetes once it is established. Clearly there is more to this type than "insulin-deprivation" or "insulin-antagonism." There are very few clues to the nature of or the onset of this kind of diabetes. In the field of experimental diabetes, Becker[23] and Bloodworth and Hamwi[27] have implicated the adrenal steroids in the production of a glomerular lesion in rabbits resembling the intercapillary lesion in man, but in no other species has it been found. Even in the rabbit no ocular micro-aneurysms have been produced like those in man.

In McCullagh's experience[15] 21 of 34 patients with Cushing's disease had diabetes and of these only three had retinopathy (two of these had hypertension and one of these was regarded as diabetic; the third was normotensive). In acromegaly, a manifest disorder characterized by excess anterior pituitary hormone, Coggeshall and Root[28] noted an incidence of glycosuria in 35 per cent and diabetes in 17 per cent. McCullagh suspects that this figure would be higher if it were based on glucose tolerance tests. In his experience 21 of 76 acromegalics had diabetes. Of these only three had retinopathy. Two of these were normotensive and one was hypertensive. He encountered none with renal failure like that seen in chronic diabetes of the conventional sort. Considering both Cushing's disease and acromegaly as disorders with surplus secretion of hormones that are "diabetogenic" it is interesting how rarely retinopathy has been encountered. I have found no reports of intercapillary glomerulosclerosis in patients with these disorders.

EFFECT OF REDUCED HYPOPHYSEAL FUNCTION

Nevertheless a few chance observations in man have recently suggested that the pituitary and adrenal hormones may be concerned in the evolution or control of these vascular lesions. Since the experimental demonstration by Houssay[29] that ablation of the anterior hypophysis ameliorates pancreatic diabetes, this phenomenon has been confirmed in man as well as its occurrence in other forms of experimental diabetes.

Of particular interest was Poulsen's observation[30] of the Houssay phenomenon in a young woman of twenty-nine. She had diabetes from the age of nine, complicated by abnormal pregnancies, and diabetic retinopathy. Following her last pregnancy she had a severe uterine hemorrhage and required a transfusion. With this last pregnancy, her insulin requirement dropped from 80-100 to 40 units daily. In the next five years she exhibited all the evidence of panhypopituitarism without further retinopathy and no hypertension. Since this report at least ten cases of amelioration of diabetes after loss of pituitary function have been reported.[31]

In 1951 Luft and Olivecrona began to perform hypophysectomy in man for a variety of diseases, among them severe diabetes with advanced vascular complications including retinopathy and renal disease. By July 1955 Luft et al.[32] reported that they had operated upon 20 such severe cases, 10 women and 10 men between 20 and 33 years of age. They had had their diabetes for 13 to 22 years and vascular complications at least 1 to 7 years earlier. Diabetic retinopathy was present in all. Traces of albumin were found in the urine in four cases, while the remainder showed a daily excretion of 0.4 to 7.5 gm. of protein. The systolic blood pressure was below 140 mm. Hg in 4 instances, between 140 and 160 mm. in 11, and over 160 mm. in the remaining 5. Diastolic blood pressure lower than 90 mm. Hg was noted in only four cases.

Of the 20 patients, 19 had been followed long enough to evaluate the effects of the operation. The twentieth had been performed too recently to be taken into consideration. Seven died within a few days to nineteen months after the operation. Of these two died within three days, two survived one month, one lived for six months, another lived for fifteen months and died in a hypertensive crisis, and the seventh died nineteen months postoperatively from renal insufficiency.

The twelve survivors evaluated with special reference to the circulatory system, renal function and eyes were followed from operation for periods of 3 to 43 months. Only one case had data to suggest that the hypophysectomy was incomplete. In 10 of these 12 cases, the preoperative systolic blood pressure was above 140 mm. Hg and above 90 mm. Hg diastolic, and fell in all save one. Only two of this group have been observed for more than a year, but both had a fall in blood pressure lasting about a year followed by a gradual rise to higher

than preoperative levels.

In nine cases heart volume was measured, and six showed diminution after the operation.

With respect to renal function in thirteen cases recorded in detail, seven showed a decline in daily urinary albumin excretion. Glomerular filtration (inulin clearance) and effective renal plasma flow (P-amino-hippurate) were measured in eleven cases. In these the effective renal plasma flow was practically unchanged but the glomerular filtration rate decreased markedly after operation and remained at this low level for the rest of the period of observation. This was attributed to the hypophysectomy and not to progression of renal disease. Similar changes occurred in hypophysectomized patients suffering from cancer without renal disease. The nonprotein nitrogen content of the blood was within normal limits before as well as after the operation.

These results seemed to show that there were no signs of progression of the diabetic renal disease in the twelve cases referred to. (It was not stated why the earlier patient who died with progressive renal insufficiency is excluded from this deduction.)

As to the ocular findings Luft and his co-workers noted no change in acuity on the whole. It is important to note that in three patients the vision in one eye *decreased* after surgery owing to acute retinal hemorrhage. In the remaining cases visual acuity either improved or remained unchanged. Subjective improvement was reported in seven. There was no change in four and decrease in vision in one.

In one patient progression of retinopathy was observed; in nine there were no signs of progression (omitting three instances of acute hemorrhage). Decrease in formation of new vessels was noted in five cases. In one patient there was marked regression of retinopathy which remained for twenty-four months of postoperative study. Luft and his colleagues concluded "that in all cases save one, except for occasional retinal hemorrhages, no symptoms or signs of progression of the diabetic retinopathy were evident. On the contrary, improvement in visual capacity and/or eye-ground changes were noted in most cases."

After hypophysectomy all patients showed increased insulin sensitivity and required between one-quarter and one-third of their preoperative daily dosage. Postoperative maintenance therapy consists of a daily dose of 5 mg. of cortisone (in three cases) and 50 mg. of desiccated thyroid. Testosterone pellets were implanted in all. DCA pellets were used at first but discontinued because DCA gave rise to hypertension and edema.

In this country Kinsell and his colleagues[26] have performed hypophysectomy in young human diabetic subjects and have likewise chosen severe cases with advanced retinopathy, nephropathy and hypertension. These workers reported their early postoperative observations in four cases and concluded that this operation will change insulin-resistant diabetes to insulin-hypersensitive diabetes which can be balanced by the judicious use of cortisone or hydrocortisone. They likewise recommended the avoidance of DCA. They suggest that the operation should not be done in the presence of advanced renal insufficiency but they do recommend the procedure for patients with progressive retinal or renal damage with diabetes which cannot be controlled by proper diet and insulin. It is difficult to tell from this report how much improvement each patient had before succumbing to other processes. In two of their patients with advanced renal insufficiency, death ensued within three to four months. In the two patients with better renal function, vision improved, hypertension receded.

These authors note the possibility of incomplete removal of anterior pituitary tissue in the present operative technics. It should be noted that the presence of extrasellar pituitary tissue may also complicate or reduce the effectiveness of the intrasellar extirpation of the pituitary. Embryonal remnants in the nasopharynx or in the migratory path of the fetal anlage may likewise occur. Whether or not these extrasellar remnants are physiologically significant in patients who do not exhibit a satisfactory reduction of anterior pituitary activity after intrasellar extirpation will require careful consideration.

Since at least three of the pituitary hormones have diabetogenic or diabetes-aggravating power (diabetauxetic—Young) it might be expected that reduction of pituitary function might have a more profound effect on diabetes mellitus than bilateral adrenalectomy. But there are insufficient cases available for comparison of the two procedures.

EFFECT OF ADRENALECTOMY

Like the Houssay phenomenon, the observation of Long and Lukens[14] in 1936 that adrenalectomy ameliorates experimental pancreatic diabetes has sustained interest in the possible beneficial effects of hypoadrenal function or reduction of adrenal tissue by surgery. They showed plainly that neither denervation nor demedullation of the adrenal protected against experimental pancreatic diabetes. (It is interesting to recall that in the late twenties and early thirties, before there was proper appreciation of the role of the adrenal cortical hormones

in metabolism, that X-ray irradiation and adrenal denervation were tried in the belief that reduction of the secretion of adrenalin might be achieved, thus reducing the glycolytic action of this substance in diabetes.)

In 1949 Green and his colleagues,[33] aware of the possible diabetogenic action of the adrenal cortical hormones, performed bilateral adrenalectomy in two stages in a twenty-eight-year-old female who had diabetes from the age of six, with an insulin requirement of 70-85 units per day. She had had hypertension (270/140 mm. Hg) from the age of twenty, signs of congestive failure, and progressive retinopathy, proteinuria and reduced renal function (maximum urine specific gravity 1.018, PSP excretion 44 per cent in two hours). Biopsy of one kidney revealed advanced glomerulosclerosis and arteriolar nephrosclerosis. Following operation, her retinal hemorrhages cleared and for the next fifteen months to the time of the published report, she held her improvement. Her blood pressure fell to normal limits and her insulin requirement to about 20 units per day.

In December 1953, Wortham and Headstream[34] reported their observations after bilateral adrenalectomy in seven diabetic subjects with hypertension and severe vascular involvement of their eyes and kidneys. After operation all patients were maintained on stabilized adrenal replacement therapy of cortisone or hydrocortisone without other adrenal steroids. Two patients observed for 14 and 10 months respectively, showed remission of vascular degeneration in their retinas, return of blood pressure to normal, decrease in proteinuria, nitrogen retention and clearance of edema. In three cases, there was no progression in pathological features 13, 10 and 8 months after operation. One died in adrenal insufficiency, two had progression of renal failure after adrenalectomy. One died of a cerebral vascular accident four and a half months after operation and the other died of pulmonary and myocardial infarcts four months after surgery. In general favorable results have occurred in inverse proportion to the severity of degenerative vascular changes.

Hamwi[35] in discussing this work reported briefly on the improvement in two diabetic subjects he had treated by adrenalectomy. He noted return of ability to work, disappearance of retinopathy and edema and improvement of the serum proteins through studies of the electrophoretic pattern and paper chromatography.

Martin and Wilson[36] have also reported an instance of bilateral adrenalectomy in complicated diabetes in a twenty-eight-year-old female with a long history of diabetes (thirteen years); complicated pregnancies occurred twice in the six years preceding surgery. In the two years prior to surgery, she exhibited progressive retinopathy, hypertension and evidence of nephropathy —presumably intercapillary glomerulosclerosis (later proved by biopsy). Following surgery it was impossible to keep her blood pressure to normal levels unless adrenal replacement therapy was minimal, and a very mild state of adrenal insufficiency was present. Her renal status would not tolerate this and there was always a prompt rise in NPN and serum potassium levels. No significant change in urinary protein was observed after operation. It is of interest that she exhibited increasing retinal "cotton wool exudates." Pigmentation increased when maintenance cortisone was increased. Blood pressure fell for one month following the operation but rose thereafter as large doses (37.5 to 50 mg.) of cortisone were given. Edema likewise persisted with this dosage. She was followed for eleven months and died with symptoms of renal and adrenal insufficiency and hyperkalemia.

Despite the fatal outcome in their patients, these authors likewise recommended that adrenalectomy in other diabetic patients with less advanced vascular and renal disease should be done in view of the present hopeless outlook in this type of patient.

ADRENALECTOMY IN NORMOTENSIVE DIABETICS WITH ADVANCING RETINOPATHY AND MINIMAL OR NO NEPHROPATHY

In commenting on the use of adrenalectomy or hypophysectomy to treat complicated diabetes mellitus with its grim prognosis Sprague[37] noted that the operative procedures had been used in cases with such advanced renal and retinal lesions that proper evaluation of their effectiveness depends on adequate testing in less advanced cases. We have had the opportunity to study two patients* who had minimal or no nephropathy but in whom advancing retinopathy or other ocular lesions were threatening to destroy vision in a remaining eye—the other eye being already sightless from earlier retinitis proliferans and secondary changes in the vitreous and lens.

In one (Case E. B.) an arrest of the retinopathy occurred following adrenalectomy and has endured fifteen months, although some advance has occurred in the concurrent mild nephropathy. The second patient (H. R. W.) has been followed for six months since adrenalectomy and has had no significant improvement; vitreous hemorrhages have continued to occur in the surviving eye, although at longer intervals than prior to adrenalectomy. The details of each follow.

*The second patient (H. R. W.) was not reported in the original Symposium paper.

CASE REPORTS

Case E. B., a white housewife of Italian extraction, age thirty at the time of adrenalectomy, had known diabetes since her third pregnancy at the age of twenty-eight. There was no family history of diabetes in siblings or her parents. Her first pregnancy, at twenty-four, was normal and she was delivered of a normal infant, after nine months gestation, weighing 7 lbs. 4 oz. Her second pregnancy, at twenty-six, terminated in the eighth month with a stillborn fetus whose weight is not known. Her third pregnancy, at twenty-eight, terminated in a spontaneous abortion at two and a half months and diabetes was discovered at this time. Occasional blood pressure readings as high as 150/76 mm. Hg were obtained at this time. She was treated for diabetes thereafter by regulation of diet and the use of 20-30 units NPH insulin each morning. Her fourth pregnancy, at twenty-nine, terminated in a miscarriage in the fifth month. Following this, in May 1953, impairment of vision became her chief complaint. Bilateral cataracts were detected and vision in each eye was found to be 20/70 uncorrected and 20/50 corrected.

In February 1954, the right fundus was described as having small hemorrhages, micro-aneurysms, and exudates. On Aug. 3, 1954, there was sudden loss of vision in the right eye due to hemorrhage into the vitreous. In the left eye, severe retinitis proliferans was detected and vision was limited to light perception.

Blood pressures between 153/86 to 134/80 mm. Hg were obtained thereafter. Albuminuria became persistent during 1953 and thereafter. Blood urea was 15 mg. per cent and urea clearance was within normal limits in October 1954. PSP excretion was in excess of 25 per cent fifteen minutes after injection of the dye in December 1954.

In February 1955, because of further impairment of vision due to repeated retinal hemorrhages in the remaining right eye, and failure to respond to massive doses of B_{12} injection, crude liver extract, the use of rutin, "adrenosem," vitamin C, and careful regulation of her diet at 1500 to 1700 calories and continued use of 20 units NPH insulin (U-40) daily with minimal glycosuria, the patient elected surgery in an attempt to conserve her remaining vision. Accordingly, adrenalectomy was performed by Dr. H. C. Maier on Feb. 24, 1955.

Preoperative preparation consisted of cortisone and testosterone administration for three days. The operation was uneventful and an intravenous infusion of 100 mg. of hydrocortisone in 5 per cent dextrose/0.9 per cent saline solution was used intermittently through the operative procedure and for the next day, with reference to the serum sodium, potassium and chlorides as well as weakness, blood pressure, pulse, and temperature for clues as to need. Cortisone acetate was given intramuscularly in doses of 25 to 50 mg. for a total of 100 mg. daily for the first two days and then reduced as blood pressure rose above preoperative levels or facial edema appeared. Until the fifth postoperative day she was still receiving up to 100 mg. of cortisone per day; by the seventh 50 mg. per day; by the eleventh, only 25 mg. per day were required. Occasional massive postoperative diuresis occurred as the cortisone was reduced. Supplemental sodium chloride tablets, 3-4 gm. per day, were likewise given until she was discharged on March 25, 1955.

Her course was remarkably uneventful until December 1955. The insulin requirement fell steadily to 5 units per day and by the ninetieth postoperative day insulin could be omitted altogether in view of the very occasional traces of glycosuria on her usual diet. Her weight remained unchanged following the operation. Her blood pressure remained stabilized between 100-120/65 mm. Hg until the tenth postoperative month when it rose to 150/90 mm. Hg.

The urinary protein before operation ranged between 3-4 gm. per day. Following operation it remained between 0.1 and 0.3 gm. per day until Oct. 4, 1955, when it rose to 2.9 gm. The twenty-four hour excretion of 17-ketosteroids was 1.1 mg. on Oct. 19, 1955. From May 16, 1955, approximately three months postoperatively, she was maintained on 0.25 mg. of fluorohydrocortisone* per day and a 1500 to 1700 calorie diet with supplements of 4 gm. of calcium gluconate daily, and no extra sodium chloride except occasional tablets (1.0 gm.) during very hot weather. Oral glucose tolerance tests performed in September with 50 and 100 gm. on two different occasions continued to show the usual diabetic pattern but without glycosuria.

The most important change, however, has been the complete arrest of fresh retinal hemorrhages and the resorption of the retinal edema and hemorrhages. Her fundi have been repeatedly examined by two ophthalmologists examining her independently. They agree (April 1956) that no fresh hemorrhages have occurred since the operation and that only scar tissue remains to impair the vision in both eyes. Retinal edema diminished and the patient reported improved vision after the first week postoperatively. The patient is happy, able to read column headlines in the newspaper, to read the numerals on a small clock, to do her own housework, and needs no assistance during her daylight activities. On refraction March 12, 1956, vision was reported as 20/100. Funduscopic examination showed inactive retinitis proliferans and no hemorrhages. The condition of this eye was definitely better than one year before. She can see television scenes and watch the movies and says, "If I can keep what vision I now have, I will be content."

While vision in the right eye improved promptly after the operation and no fresh hemorrhages have been observed since, evidence of increased albuminuria and the return of hypertension were observed ten months postoperatively. In January 1956 (eleventh postoperative month) she suddenly developed fulminating streptococcal pharyngitis with a temperature of 105 degrees and prostration. Massive doses of penicillin and increased dosages of cortisone checked this infection in 3-4 days. No hematuria developed but microscopic evidence of granular casts and increased numbers of leucocytes persisted following this infection.

No facial edema developed but within the four months preceding this infection and lasting a few weeks after it, this patient exhibited fusiform swelling of the proximal interphalangeal joints of the fingers of both hands without pain or erythema, but had some difficulty in closing her hands tightly. This transient episode of arthropathy occurred while she was being maintained on 0.25 mg. of fluorocortisone alone and subsided when cortisone was resumed.

In the period 6 to 12 months after the operation while being maintained on 0.25 mg. fluorocortisone per day her skin

*I am indebted to Dr. Henry A. Strade of E. R. Squibb & Sons for the supply of fludrocortisone acetate (9-α-fluorohydrocortisone) for both patients reported here.

became darker and she exhibited melanotic pigmented spots in the buccal (mucosa) and vaginal mucosa. Since changing to cortisone in a daily dose of 12.5 mg. the pigmentation has been visibly fading.

Following the streptococcal infection in January 1956 her blood pressure continued elevated. When examined at two-week intervals in March 1956 blood pressure was 170/110 and 154/92 mm. Hg respectively and proteinuria was still marked (7 gm./day). Because of this elevation fluorocortisone was stopped and the patient has been maintained since on 12.5 mg. of cortisone per day. Since March 15 her blood pressure has fallen to normal levels again (110-126/76-80 mm. Hg) and proteinuria has receded somewhat (3.7 gm./24 hrs.) and glycosuria and the insulin requirement have been nil.

Case II, H. R. W., a thirty-nine-year-old white male, a mechanical engineer, was known to have diabetes since the age of fifteen. It was detected while he was in a hospital convalescing from a fracture of a leg, acquired in a tobogganing accident. The patient was variably controlled by diet and insulin thereafter. He exhibited great intellectual capacity as a student, but has always been moody and on occasion has exhibited agitated compulsive behavior sometimes associated with depressed states, sometimes with abnormally euphoric states. Diabetes was difficult to regulate in these periods but he had no episodes of coma except one briefly in his sixteenth year. His vision began to fail in his left eye in October 1952 at age 35 with retinal hemorrhage and detachment and he became blind following further retinal detachment in this eye in 1953. Repeated hemorrhages occurred in the right eye beginning about October 1953. As vision began to fail in this eye too during the spring of 1955, his physician advised him to consider the possible benefit of adrenalectomy.

Before operation, tests of renal function disclosed a blood urea nitrogen of 14.8 mg. per cent. Urinalysis disclosed specific gravity 1.024 without glucose, and no formed elements; proteinuria did not exceed 0.1 gm. per liter. Other laboratory data included serum sodium, potassium, chlorides, hemoglobin, erythrocytes and leucocytes, in normal ranges. Cholesterol measured 209 mg. per cent and cholesterol esters 156 mg. per cent. The preoperative excretion of 17-ketosteroids in twenty-four hours was normal (13.7 mg. in forty-eight hours), and the average twenty-four hour loss of glucose in the urine was 2 per cent per liter. An electrocardiogram (12 leads) was within normal limits. Intravenous pyelography and radiograms of the chest were likewise normal; the cardiac silhouette was not enlarged.

There were no abnormalities disclosed by the physical examination except for the following changes in the eyes: The left eye was blind because of sclerosis of the lens secondary to the old retinal detachment and vitreous hemorrhages. Vision in the right eye was 20/200. The pupillary function was normal. There was a slight central haze in the lens. The vitreous was hazy and contained fresh blood amid many floating dense black particles. The central area of the fundus was too hazy to visualize. The periphery showed no retinal abnormalities.

Because of continued hemorrhages into the vitreous of his functioning right eye followed by progressive impairment of vision, this patient elected adrenalectomy in an effort to check the retinopathy and better control his diabetes.

Bilateral adrenalectomy was performed on Nov. 17, 1955, and normal adrenal glands were removed by Dr. H. C. Maier without incident. Preoperative preparation included priming with 100 mg. of cortisone acetate and 100 mg. of testosterone propionate (intramuscular) per day for two days. Hydrocortisone (100 mg.) in saline infusion was given intermittently for two days postoperatively with reference to urine volume, serum sodium, potassium and chlorides, blood pressure, weakness and pulse, to determine rate of infusion. Biopsies were taken of both kidneys and multiple sections disclosed no abnormalities and no histological evidence of intercapillary glomerulosclerosis.

His postoperative course was peculiar in that it soon became apparent that his insulin requirement was not greatly reduced. In the first two weeks during reduced caloric intake there was apparent insulin sensitivity with rapid reduction of blood glucose and occasional hypoglycemic symptoms after small increments (4 to 6 units) of regular insulin given according to the degree of glycosuria. But when he was able to take a general diet of about 2000 calories per day his insulin requirement matched his preoperative needs of 50 units per day in divided doses. The completeness of the adrenalectomy was established by the fall of 17-ketosteroid content of a twenty-four hour urine collection. From a preoperative level of 6.8 mg. in twenty-four hours it was 4.8 mg. on the fifth postoperative day and 4.1 mg. on the thirtieth postoperative day, and chromatography* demonstrated that most of this was androsterone. Preoperative dehydroanisoandrosterone was 1.7 mg. in twenty-four hours, while the postoperative specimen contained none.

This patient's requirements for replacement adrenal steroid therapy have been relatively high. Postural hypotension and increased water and sodium loss with weight loss occurred unless he received 0.25 mg. fluorocortisone per day or 25 mg. of cortisone acetate supplemented by 0.1 gm. of fluorocortisone. On fluorocortisone alone ketoacidosis appeared repeatedly in this patient and larger amounts of insulin to reduce glycosuria were needed than when he took cortisone alone. It is probable that his continued high insulin requirement (*ca* 50 units per day) is related to the large amount of adrenal steroid found necessary to prevent weakness, postural hypotension and adequate serum electrolytes.

Ten days after the operation this patient reported a fresh hemorrhage in his functioning right eye and since that time, at successively longer intervals there have been occasional recurrences, one or two per month, which clear spontaneously. Vision has diminished further with increasing haziness of the vitreous and slight improvement with the resorption of the hemorrhage. With the continued decline of vision the patient has found it necessary to begin the study of Braille in order to continue his usual intellectual pursuits.

COMMENT

It is interesting that in Case I (E. B.) retinopathy appears checked while nephropathy has continued. In view of the positive biopsy for intercapillary glomerulosclerosis, and the recent episodes of increased proteinuria and hypertension it must be acknowledged that adrenalectomy has not favorably influenced the neph-

*Courtesy of Dr. Seymour Liberman, College of Physicians and Surgeons, Columbia University, New York, New York.

ropathy in this patient. Further follow-up studies will be needed to determine the ultimate effects if any.

In Case II (H. R. W.) without nephropathy to begin with, retinopathy in the form of repeated hemorrhages into the vitreous has continued. More time is necessary to evaluate the full effects of adrenalectomy in both subjects. The failure to reduce the insulin requirement in the second subject (H. R. W.) likewise reiterates the variegated basis for the disturbances in carbohydrate metabolism in diabetes. It suggests that in this subject the disturbances leading to repeated hemorrhages in the vitreous after their removal are not necessarily mediated via the adrenal glands, as has been suggested by others, unless the maintenance dosage of cortisone and fluorocortisone is related to the continued hemorrhages.

It is to be hoped that some method less radical and more hopeful than adrenalectomy or hypophysectomy will be found to arrest or prevent the vascular complications represented by these patients. Anti-adrenal or anti-pituitary agents may achieve "medical adrenalectomy" or "medical hypophysectomy" by reducing the secretion of those hormones or substances which may aggravate or contribute to the diabetic state. The recent induction of pantothenic acid deficiency in man[38] with evidence of reduced adrenal cortical function, and the demonstration[39, 40] in the alloxan diabetic rat that pantothenic acid deficiency may reduce the glycosuria, give some hope that methods may be found to reduce the adrenocortical activity in various disease states including diabetes. Whether or not new and better methods of irradiation of the pituitary or the hypothalamus may be helpful in the problem discussed is also worthy of further investigation. The outlook for patients like those reported here unfortunately continues to be grim. More studies of more such patients are needed for critical evaluation of the effectiveness of hypophysectomy and adrenalectomy in modifying the course of diabetes with disseminating capillary and arteriolar disease. While considerable reduction of insulin requirement may follow hypophysectomy, adrenalectomy or adrenal insufficiency, the therapeutic use of supportive adrenal steroids may have interfered with the expected fall. Insulin sensitivity apparently varies with each case and may be only slightly increased in some patients even after adrenalectomy.

SUMMARIO IN INTERLINGUA

Function Hypoadrenal e Adrenalectomia in Diabete Human

Le relation del function adrenal a diabete mellite es revidite con referentias special al effecto de reducite function debite a sia morbo glandular, sia adrenalectomia, o sequente un reduction del function hypophyseal.

Le datos nunc disponibile non corrobora definitivemente le opinion que le complicationes vascular que affice le vasos retinal o le arteriolas del ren o le capillares glomerular es influentiate beneficamente per le reduction del function adrenocortical.

Es reportate duo casos additional de complicate diabete mellite que esseva tractate per adrenalectomia. Ambe patientes habeva perdite le vision de un oculo. Ambes habeva progressive retinopathia que reduceva le vision del altere oculo. In ambe casos biopsias renal esseva executate. In un caso le biopsia renal revelava precoce glomerulosclerosis intercapillar, associate con minime grados de hypertension e leve albuminuria. In iste patiente le retinopathia se arrestava dece-cinque menses post le operation, sed le nephropathia persisteva. Le secunde patiente ha essite sub observation postoperatori durante sex menses. Le retinopathia non es arrestate, e le vision del oculo remanente ha continuate decrescer.

Le prognose pro diabeticos con retinopathia e nephropathia remane triste. Sed studios additional del effecto de reducite function adrenal in minus avantiate casos es requirite pro un evalutation critic del utilitate de iste forma de tractamento.

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DISCUSSION

ABBIE I. KNOWLTON, M.D., (*New York*): Dr. Graef has given us a very comprehensive summary of clinical data relating to the action of the adrenals and of the pituitary upon carbohydrate metabolism. He has reviewed in detail the results that have been obtained by ablation of either the adrenals or the pituitary in patients with diabetes, and has added a most interesting case of his own. (*Editor*: *The second case* (H. R. W.) *referred to in the text was not presented at the Symposium.*)

It seems to me that there are two arguments possible for advocating either adrenalectomy or hypophysectomy in diabetes. The first is the argument that these glands

exert effects upon carbohydrate metabolism which are antagonistic to the action of insulin, hence the removal of either of them should reduce insulin antagonism. The initial hypophysectomies that were done in diabetics back in the thirties were done on this premise and there is no question that insulin requirements have been reduced posroperatively. However, I think it is well to point out that even in a completely adrenalectomized individual, severe diabetes can exist. In 1949, Dr. Kritzler and I reported such a patient: a young woman who developed her diabetes subsequently to the onset of Addison's disease. Although like most Addisonians, she had a very small daily requirement for insulin (in the neighborhood of 8 units), when insulin was withheld she excreted over a hundred grams of carbohydrate in twenty-four hours.

The second argument which has been proposed for the removal of the adrenal or of the hypophysis is based upon the hypothesis, presented by Becker initially, that the gluco-corticoids have a quite specific role in the development of certain of the complications of diabetes. I should like to spend just a few minutes of this discussion presenting some of the evidence which argues against this view.

The similarity of the renal lesions which have been produced experimentally in rabbits by cortisone or hydrocortisone and alloxan to those described by Kimmelstiel-Wilson in humans has been questioned, and although retinal lesions have been seen in rabbits given alloxan and ACTH this has not been reported in animals treated with cortisone.

The second argument, I think, against the gluco-corticoids playing a specific role is one which Dr. McCullagh has already mentioned, and which Dr. Jailer also referred to this morning. This is the fact that the occurrence of retinopathy is relatively unimpressive in the patients with Cushing's syndrome. If the hypothesis were correct, certainly these patients would be most likely candidates for extensive retinopathy.

In some forty-seven patients with Cushing's syndrome seen at the Columbia Presbyterian Medical Center, only five had significant degrees of retinal damage. Dr. McCullagh pointed out the fact, however, that we often see patients with Cushing's disease when they have not had their disease present for a matter of years. Of the 47, some 14 had had more than five years of symptoms prior to coming to the hospital. Five of these 14 were frankly diabetic, and in all the group hypertension was the rule. In spite of this, in these 14 only 3 had significant vascular changes in the eyegrounds.

The third argument, it seems to me, against the specific role of the gluco-corticoids is suggested by the course of those patients who have been adrenalectomized. Improvement in the eyeground changes or arrest of lesions has at times been seen while these patients have been maintained on gluco-corticoids as their primary adrenal replacement therapy. The actual amounts used have varied, in the instances reported, from some 20 to 50 mg. of hydrocortisone or of cortisone daily. If we consider the recently published data of Silber that the normal adrenal secretes in the neighborhood of 22 mg. of hydrocortisone a day, and if we consider that Dr. Pearson's patients following hypophysectomy are maintained in quite normal carbohydrate balance on doses of cortisone of this same order of magnitude, I should like to suggest that these patients following adrenalectomy have received sufficient cortisone to keep them euadrenal with respect to gluco-corticoids. In fact, the gluco-corticoids are the only adrenal steroids in which they are not insufficient following adrenalectomy. And yet, as Dr. Graef has pointed out, it is certainly true that certain of these patients have shown improvement in their eyegrounds.

If improvement is not due to reduction in the gluco-corticoids, to what can we ascribe it? The answer to this question is certainly not obvious. I was interested in looking over the case of Green, Nelson, and Dodds and those cases of Wortham and Headstream who showed improvement, that the most striking visual changes occurred in patients who showed the most marked drop in blood pressure following operation. I have wondered whether a considerable proportion of the visual improvement might not have been a nonspecific one related to the effect of the operation indirectly on the blood pressure and whether perhaps the same type of thing might have been produced, say, by sympathectomy.

I am sure this is not the whole answer, as witness this patient of Dr. Graef's who never had significant degrees of hypertension preoperatively and yet postoperatively has shown at least arrest of the retinal lesions. From the point of view of aiming for reduction in gluco-corticoids this patient has been maintained in a manner which would seem most logical. Her only steroidal replacement has been 9-alpha-fluorocortisone, which, at this daily dose of a quarter of a milligram, should have a negligible effect upon carbohydrate metabolism.

HAROLD RIFKIN, M.D., (*New York*): Dr. Graef has discussed the clinical and experimental observations which suggest that adrenal cortical hyperfunction may be involved in the pathogenesis of diabetic retinopathy and nephropathy. Previous attempts to evaluate the level of adrenal cortical activity in diabetic patients, based on crude measurements of urinary ketosteroids or corti-

coids, have yielded discordant results, the outputs ranging from abnormally low to normal to abnormally high values.

In view of these conflicting data, and since total hypophysectomy and bilateral adrenalectomy have already been and are continuing to be performed for possible alleviation of diabetic degenerative vascular disease, we have, in association with Dr. Seymour Lieberman and his colleagues at the College of Physicians and Surgeons, attempted to reassess adrenal cortical function with newer available technics. It is conceivable that the older methods employed by previous investigators for the estimation of the urinary metabolites of the adrenal hormones were inadequate for the detection of subtle evidences of adrenocortical hyperactivity. This investigation has been carried out in patients who have been carefully evaluated with regard to the existence, nature, and extent of vascular lesions, since no attempt was made in most of the previous studies to distinguish uncomplicated diabetic patients from those with retinopathy, nephropathy, and other signs of vascular disease.

The question which confronted us was how to obtain objective evidence of adrenal activity in a disease state, not characterized by the usual clinical criteria associated with adrenocortical hyperfunction or hypofunction. Certainly the eosinophil count is not specific enough; measurements of total urinary ketosteroids or corticoids by routine clinical technics are inadequate, probably because they cannot detect subtle differences. It seemed that probably the most direct approach would be to measure the individual urinary metabolites arising from the secretory products of the adrenal cortex. These are the 17-ketosteroids and the corticosteroids; of these two alternatives, only the measurement of the 17-ketosteroids by a satisfactory method was available to us when this study was begun two years ago. This technic permitted the estimation of the 17-ketosteroids excreted in a 24- or 48-hour urine in a manner which was both reproducible and amenable to a fairly large scale clinical investigation. Possibly measurements of the more abundant individual urinary corticoids such as compounds E, F, Tetrahydro E, Tetrahydro F, Cortol and Cortolone would have afforded us a better indication of adrenocortical activity. Although we are now in the process of studying these urinary metabolites, at the onset of our investigation no adequate method for the isolation of these corticoids was readily available. Our discussion today is therefore limited to data, not quite complete, on the excretion of the individual ketosteroids in: 1) a series of normal patients, 2) patients with uncomplicated diabetes, 3) patients with diabetic retinopathy and diabetic nephropathy, and 4) in nondiabetic patients with various renal disorders, in varying degrees of azotemia. The blood corticoids, both free and conjugated, have been estimated in a number of these patients through the kindness of Dr. Alfred Bongiovanni of the Children's Hospital in Philadelphia. The effects of ACTH on the blood corticoids and individual urinary ketosteroids in a selected group of these patients have also been observed. To assure the homogeneity of groups, and to allow comparison of the results obtained, factors such as age, sex and duration of disease were controlled by appropriate selection. Renal function was estimated by serial determinations of the BUN, serum creatinine, and endogenous creatinine clearance; proteinuria was estimated quantitatively in each 24-hour urine sample simultaneously analyzed for ketosteroids.

RESULTS

Each urine was hydrolyzed by three different methods. The combined neutral extracts from these three hydrolyses were separated by Girard's reagent, yielding a neutral ketonic extract. This ketonic fraction was resolved into its components by gradient elution chromatography on alumina. The ketosteroids isolated in this way were identified by infrared and ultraviolet spectroscopy and were estimated by the Zimmermann reaction. For convenience sake, the C-19 17-ketosteroids can be separated into two groups, those which have an oxygen at the 11 position, and those which do not. These will be referred to hereafter as the C-19-11 oxy and C-19-11 desoxy compounds respectively. The C-19-11 desoxy compounds, such as dehydroisoandrosterone, isoandrosterone, androsterone and etiocholanolone, arise from steroid precursors which have their origin either in the adrenals or gonads. To contrast, the C-19-11 oxy compounds, such as 11 ketoetiocholanolone, 11 O H androsterone, and 11 O H etiocholanolone, appear to be derived solely from adrenal precursors, principally compound F.

Examination of our data thus far reveals: 1) Patients with uncomplicated diabetes excrete total identified ketosteroids in approximately normal amounts. Diabetic patients with retinopathy and nephropathy excrete quantities of ketosteroids which are either normal or somewhat decreased. In contrast, all nondiabetic patients with renal insufficiency excrete significantly decreased amounts of total ketosteroids. 2) When these substances are broken down into the two categories of compounds, the 11 desoxy and the 11 oxy metabolites, it becomes apparent that patients with diabetic vascular disease and

nondiabetic renal disease excrete smaller amounts of C-19-11 desoxy compounds than do the normals or uncomplicated diabetics. 3) It is further apparent that concentrations of C-19-11 oxy compounds in all three diabetic groups are within normal limits in contrast to the low excretion of these substances in nondiabetic renal insufficiency. 4) In view of the extensive renal insufficiency characteristic of patients with diabetic nephropathy and mild to moderate renal insufficiency present in our patients with retinopathy, it seems more reasonable to compare the excretion pattern of these diabetic patients with that found in patients with nondiabetic renal disease rather than to compare them with normal subjects. It is possible that perhaps the greater degree of azotemia or proteinuria in patients with nondiabetic renal disease could account for these differences.

In two groups of patients with diabetic and nondiabetic nephropathy, of approximately the same age and sex, with a similar degree of renal insufficiency as established by the methods employed, and with similar quantities of proteinuria—yet, the concentration of C-19-11 oxysteroids in the patients with diabetic nephropathy exceeds the amounts found in the nondiabetic renal patients. More precise studies of renal hemodynamics and clearances of free and conjugated corticoids in these patients would perhaps be helpful to explain the observed differences which might simply be related to a differential or selective renal excretion of these compounds.

The more imminent problem now confronting us is whether the normal concentrations of C-19-11 oxysteroids noted in the urine of patients with diabetic vascular disease, in spite of extensive renal insufficiency, can be related to adrenocortical hyperactivity. To obtain further evidence of adrenal hyperfunction, measurements of both free and conjugated corticoids were performed on plasma obtained from a number of these patients. No significant deviation from values found in control subjects was noted. This finding, as is well-known, is still not incompatible with increased adrenal function. When ACTH was administered in 40 mg. doses intravenously to several patients with diabetic retinopathy and nephropathy, the blood corticoids, the total identifiable ketosteroids, and the individual urinary ketosteroids rose in a manner quite indistinguishable from that observed with normal controls.

To summarize our findings then: Diabetic patients with retinopathy and nephropathy have been found to excrete normal amounts of C-19-11 oxysteroids. When considered in the light of the impaired renal clearance of these patients, these so-called normal amounts may be considered to be excessive. When these excretion levels are compared with those of patients with nondiabetic renal disease of similar degree, this observation becomes still more suggestive of adrenal hyperactivity. It is quite true that this observation may simply reflect a selective difference in the manner in which the kidney handles this class of steroids, although no such selective mechanism for the excretion of any group of steroids has ever been described.

Suppose the findings which we have noted do reflect moderate hyperactivity of the adrenals, of what physiologic or etiologic significance is this? Can small increases in the secretion of Compound F and related compounds be related to the vascular complications? It is, of course, possible that the end-organ response in diabetics is so altered that slightly increased or even normal quantities of adrenal secretory products can be significant. On the other hand, it is conceivable that measurements of the known and recognizable adrenal hormone metabolites such as we are making are merely reflections of other adrenal processes, possibly not even related to hydrocortisone synthesis, and these unknown factors are related to the development of diabetic vascular disease.

Bilateral adrenalectomy or hypophysectomy might then be considered: 1) if there is a true hyperactivity of the adrenal gland, 2) or even if the normal secretion of adrenal hormonal metabolites is damaging to unusually sensitive end-organs such as the retinal and glomerular capillaries. Although our data are suggestive for either of these concepts, we believe that further information should be accumulated along the lines which we have previously outlined before considering these surgical procedures as a routine measure for the prevention or alleviation of diabetic retinopathy and nephropathy.

RACHMIEL LEVINE, M.D., (*Chicago*): My purpose is not to discuss the papers that were so ably presented. I simply would like some information.

One question was suggested by an unusual patient who came to her physician and was discovered to have very advanced diabetic retinopathy, typical, without any signs or symptoms of diabetes or any previous history of diabetes. The glucose tolerance was normal, and so were the 17-ketosteroids and the 11-oxysteroids. The blood pressure was not increased. The only positive finding in relation to diabetes was a very severe history of diabetes in her family—two sisters, her mother and her father. But she herself did not suffer from overt diabetes, but has advanced bilateral diabetic retinopathy and some albuminuria, although one cannot call it definitely a Kimmelstiel-Wilson syndrome. This is just an

item for elucidation.

The second question relates to pancreatic diabetes. Has anybody followed, for a sufficiently long period of time, patients who are diabetic because of total pancreatectomy, and do such diabetics develop typical lesions in the retina and in the kidney?

E. PERRY MCCULLAGH, M.D., (*Cleveland*): I can't answer Dr. Levine's question fully, but he has pointed out an interesting aspect of diabetes that is important to the clinician. Clinicians who see a great many patients having diabetes are well aware of the fact that retinopathy may be associated with very mild diabetes—at least to this extent: Diabetic patients sometimes show no glycosuria on a random test, and may show no hyperglycemia on a random blood sugar test. Under such circumstances, diabetes mellitus may have been diagnosed by the ophthalmologist. However, all of such patients whom I have examined have had abnormal glucose tolerance.

As far as the question of diabetic retinopathy after pancreatectomy is concerned, we have had the good, or bad, fortune to have cared for about a dozen patients after total pancreatectomy. Our surgeons are extremely discouraged with the results of that operation. All but two of our patients were operated upon for carcinoma of the pancreas. Of the total group with carcinoma, one, who we thought might be going to get by without metastasis, was examined last week and was found to have a mass in the upper abdomen. The two patients who did not have carcinoma but had extensive disease of the pancreas were recently operated upon, and the results cannot yet be fully evaluated. None had retinopathy.

I think, as Dr. Pollack mentioned to me, that Dr. Randall Sprague once reported a patient in whom the diabetes which followed pancreatectomy was associated with diabetic retinopathy.

A Plan for Later Years

What about the effect of environment on the senior citizen's emotional responses? Perhaps the most devastating attack on the ego is the matter of forced retirement. It is unfortunate that industry has arbitrarily set the age of 60 to 65 as the time at which retirement becomes compulsory. When one feels fit and has the desire and capacity to work, it is a serious blow to the ego to be put on the shelf and branded as useless by society. In order that inactivity may not become wearisome and engender dissatisfaction, definite and constructive plans must be worked out, preferably long before retirement is imminent; otherwise his feeling of uselessness leads to anxiety, irritability, and tensions which cause discomfort not only for the individual but for those of his immediate environment as well.

H. A. Steckel, M.D., from the article "Emotional Aspects of the Aging Process," *New York State Journal of Medicine* 55:3605-06, Dec. 15, 1955.

EDITORIALS

MEASUREMENT OF INSULIN ACTIVITY IN BLOOD

A reliable and accurate method for the estimation of the insulin concentration in the blood should prove useful for the elucidation of many problems in metabolism.

In the last few years, the technical aspects of this assay procedure have been investigated and two methods are now available, one in vivo, the other in vitro.

The in vivo method depends on the hypoglycemic effect of insulin. The test animals used have been prepared in different ways to increase their sensitivity. Gellhorn et al.[1] used hypophysectomized-adrenodemedullated rats and found that 200 microunits of insulin gave a small but significant lowering of the blood sugar with this preparation. In 1947 Anderson et al.[2] prepared hypophysectomized-adrenodemedullated rats, previously rendered diabetic with alloxan to prevent the possibility of variation in the rate of secretion of endogenous insulin.

Bornstein,[3] using a modification of the above technic, obtained significant effects with insulin doses as low as 50 microunits. He prepared alloxan-diabetic, hypophysectomized-adrenalectomized rats. Yenerman et al.[4] used alloxan-diabetic hypophysectomized mice, obtaining a significant effect down to 10 microunits.

The technical difficulties involved in the preparation and maintenance of these animals, and the actual conduct of the assay are considerable. A relatively simpler in vitro method has been in use during the last four years.[5, 6, 7]

In this in vitro method, the isolated rat diaphragm is used. The method depends on the fact that small amounts of insulin increase the utilization of glucose by the isolated rat diaphragm[8] and that a quantitative relationship exists between the concentration of insulin in the incubation medium and its effects on the glucose metabolism of the diaphragm.[9, 10] By rigorous control of the experimental conditions, this method can be used for the estimation of plasma-insulin activity, or the effective plasma-insulin concentration, i.e., the sum of insulin and its synergists, if any, on the one hand and its antagonists on the other. It is of course true that the proof of this contention is not absolute but this applies equally to the identification of the substance measured in many bioassays. Suffice to say that, in our present state of knowledge, there is no other hormone besides insulin which will, per se, cause an increased glucose uptake by the diaphragm except growth hormone. This latter has an insulin-like effect under special experimental conditions[11] which do not ordinarily pertain in the above assay procedure. Moreover, the substance estimated by this method has many attributes in common with insulin; e.g., it disappears from the plasma of depancreatized dogs[5] and cats,[12] it is found in increased amounts in the plasma of normal fasting subjects after the intravenous injection of insulin,[13] it is considerably higher than normal in patients with severe spontaneous hypoglycemic attacks, falling into the normal range after the removal of islet-cell tumors which were found at operation and shown histologically to be composed entirely of β-cells.[14] Also the increased glucose uptake from standard insulin solutions and from plasma can be inhibited by cysteine and glutathione,[5, 6, 7] and insulin loses its activity in the presence of such SH compounds.

As can be seen from the accompanying table, the values for plasma-insulin activity in fasting animals and man, whether estimated by the in vivo or in vitro method, have varied around 100 microunits/ml. In normal human subjects, one hour after 50 gm. glucose by mouth, there is usually a considerable increase, up to eight-fold in plasma-insulin activity above the fasting level.[6] However, one investigator,[7] using the rat diaphragm technic has found very much higher values for plasma-insulin activity in normal subjects 2½ hours after glucose by mouth. Moreover, it was observed that this plasma, assayed by the in vitro method to contain 13,000 microunits/ml., had no effect when tested in vivo upon the blood sugar of alloxan-diabetic hypophysectomized rats. By contrast, 2,000 microunits of insulin produced a highly significant fall of blood sugar in the same rats under similar experimental conditions. The cause for this discrepancy is not yet clear, for the in vitro method used by the several investigators in this

TABLE 1

Insulin-activity of plasma, serum and whole blood in normal human subjects and in animals

Investigators	Method*	Test Material**	Insulin activity (microunits/ml.)	Species
Gellhorn et al.[1]	h.e. in HA rats	W	± 200	Man, 2 hours after food.
Gellhorn et al.[17]	h.e. in HA rats	W	± 100	Dog, fasted 24, and rabbit.
Anderson et al.[2]	h.e. in ADH rats	P	< 100	Rat, fasted 17 hours.
Bornstein et al.[3]	h.e. in ADHA rats	P	100	Man, fasting.
Bornstein et al.[3]	h.e. in ADHA rats	P	240-400	Man, 2 hours after 50 gm. glucose orally.
Perlmutter et al.[18]	R.D.	P	< 5,000	Man.
Groen et al.[5]	R.D.	S	50-500	Man and dog.
Vallance-Owen et al.[6]	R.D.	P	40-80	Man, fasting.
Vallance-Owen et al.[6]	R.D.	P	100-800	Man, 1 hour after 50 gm. glucose orally.
Vallance-Owen[12]	R.D.	P	± 100	Cat, fasting.
Randle[7]	R.D.	P	± 13,000	Man, 2½ hours after 50 gm. glucose orally.

*R.D.=rat diaphragm
h.e.=hypoglycemic effect
HA=hypophysectomized adrenodemedullated
ADH=alloxan-diabetic hypophysectomized
ADHA=alloxan-diabetic hypophysectomized adrenalectomized

**W=Whole blood
P=Plasma
S=Serum

field is essentially similar, though varying somewhat in practical detail.

Turning now to the studies made in diabetics, interest has been centered on the two broad clinical types of diabetic patients. In the first, the patients, besides having hyperglycemia and glycosuria, usually develop ketosis and rapidly lose weight unless given insulin injections. In the second type, the patients do not require insulin and are usually obese middle-aged females. They have hyperglycemia and glycosuria but rarely develop ketosis unless their diabetes is complicated by infection and they generally recover from the diabetic state when they lose weight on a low carbohydrate reducing diet.

Bornstein and Lawrence,[15] using the in vivo method, found that the first group had no insulin activity in their plasma when untreated, whereas the second or obese group had almost normal amounts of circulating insulin. Similar results have been obtained using the in vitro technic.[16] In uncontrolled but nonketotic diabetics who require insulin, no plasma-insulin activity was found. Moreover, when insulin was added to the plasma of these patients in vitro, its activity was apparently inhibited. When the patients in this group were controlled, however, plasma-insulin activity was found essentially in the normal range, and now the activity of added insulin was not diminished. In other words, the presence or absence of this apparent inhibition seemed to depend on the amount of exogenous insulin injected; provided enough is given to lower the blood sugar to physiological levels, then the inhibition is overcome. In the obese group, insulin activity was found, essentially in the normal range, and when a known amount of insulin was added to the plasma of these patients in vitro, its activity was not diminished.

The nature of this apparent inhibition in the plasma of insulin-requiring diabetics is at present being investigated by the author, using Houssay and Long-Lukens animals both alone and with hormonal replacement.

In this way, the relative importance in human diabetics of hypoinsulinism or of hypersecretion of the hormones antagonistic to insulin action may ultimately be ascertained.

Correlation of plasma-insulin studies with those of insulin concentrations in the pancreas, especially if the latter can be expressed in terms of islet volume, would give a better knowledge of the significance of changes in the amount of insulin in the islets.

It is perhaps important to emphasize that the measurement of plasma-insulin activity only reveals a part of the over-all picture of insulin metabolism. It indicates the amount of insulin available for the tissues. Thus it will be appreciated that, as with glucose, the quantity actually utilized at the cellular level in different conditions may not bear any direct relationship to the concentration in the blood.

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Antoine Laurent Lavoisier

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The French political revolution occurred simultaneously with another revolution, that is, a scientific one. The political revolution delayed the progress of the scientific one by causing the death of one of the greatest minds of that century.

In the history of science before the twentieth century three great revolutionary periods stand out. The first was initiated by Newton's publication of *Mathematical Principles of Natural Philosophy* in 1687. The second began in 1789 (the year of the fall of the Bastille) with the publication of Lavoisier's treatise, *Elements of Chemistry,* and the third was occasioned by Darwin's *Origin of the Species* in 1859. Lavoisier changed the entire outlook of chemistry.

Antoine Laurent Lavoisier was the son of a prosperous French merchant. He was born on Aug. 26, 1743. His early education was excellent, and later he was trained in the law. He developed an interest in politics which remained with him for the rest of his life. He used his political position to institute many social reforms in the life of the oppressed French peasant.

Among his achievements he demonstrated the importance of scientific farming. He created credit bureaus to relieve the burden of debt, and spent much of his own money in these ventures. Correspondence found in his desk bears out his extensive world-wide acquaintanceship. Among his correspondents were Benjamin Franklin, Cavendish, Black, Priestley, and Scheele in Sweden.

On May 8, 1794, he was guillotined at the order of the Revolutionary Tribunal, a fate that he had anticipated.

During his early life the phlogiston concept was the

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accepted philosophy. The four element theory had been expounded at least 400 years before the birth of Christ and was still being taught in the universities as late as the early nineteenth century. This theory was very simple—all substances were composed of four elements, earth and water, air and fire. It was Lavoisier in the last third of the eighteenth century who showed that substances burn by combining with the element oxygen, not by releasing fire-stuff.

It is true that Boyle had known of oxygen and that Priestley and Black in England also were experimenting along these lines. Cavendish had discovered the composition of water at about the same time as Lavoisier. What made Lavoisier great was his ability to recognize the importance of these discoveries. Priestley merely called his product "de-phlogistinated air" and tried to fit his discovery into the old four element theory. Lavoisier built a whole new concept of chemistry and physiology.

Lavoisier is generally accepted as the father of the present day science of metabolism. The lineage is easy to trace. His pupils were Berthollet and La Place. They in turn had as one of their pupils Gay-Lussac. From Germany came Liebig to work with Gay-Lussac and bring back to his native country the stimulation of these brilliant imaginative minds. Liebig's pupil was Voit. Graham Lusk went to Germany to study with Voit and returned to these United States with the accumulated experiences of the German school. Lusk served as the stimulating focus for the greater part of the early development of the field in this country.

One day, while browsing in a London book shop, I chanced across the *Medical Commentaries* for the year 1785 (Vol. X), edited by Andrew Duncan, M.D. The index attracted my attention. It listed an account of new books by Abbe Spallanzani, M. Lavoisier, Thomas Fowler, M.D., and William Withering, M.D., and a series of original medical observations.

The reviewer of M. Lavoisier's book, titled *Experiences fur la Respiration des Animaux, et fur les Changements qui arrivent a l'air en passant par leur Poumon,* recognized the importance of what he read. He pointed out that while Priestley had described the properties of oxygen a few years previously, his interpretation was not entirely correct. Lavoisier showed that mercury when heated in air removed a part of the air and the residue ceased to be fit for respiration or combustion, and that this residue did not render limewater turbid. When this calcined mercury was heated the gas given off restored the air to all of its former properties. He further showed that when an animal is placed under a bell-glass it perishes in a short time. The remaining air had the properties of the air remaining after calcining mercury as described above. It had one further property; it rendered limewater turbid. Furthermore he showed that he could restore the life-maintaining power of the air exhausted by an animal by adding to it the "vital air" recovered from heating the calcined mercury. He concluded that "while calcination of mercury produces no other change on atmospheric air than that of depriving it of vital air, the respiration of animals besides this effect produces in the atmospheric air a portion of fixed air." After performing various other experiments with charcoal burning in the air he further concludes, "that during animal respiration there is an absorption of vital air which is converted in part in the body to fixed air which is excreted by the lungs."

From these early experiments Lavoisier developed the concept of the importance of oxygen. It was he who gave this element its name. He proposed the concept that life depended on a series of oxidative processes with the resultant production of heat. By a series of ingeniously devised experiments he measured the amount of oxygen consumed by man and animal during rest, after eating, when exposed to cold, and during a period of work and exercise. The values he found with his crude melting ice calorimeter are in strict accord with modern day concepts. Lavoisier demonstrated to his own satisfaction that perspiration regulated the heat lost from the body and that digestion replenished the blood with materials.

Today the technics have been refined; instead of weighing melted ice water, electronic gadgets are employed. The newer results are more accurate but the basic principles remain those developed by Lavoisier from 1770 to the time of his death in 1794.

ABSTRACTS

Berry, Robert E. L.; and Flotte, C. Thomas: PERIPHERAL ARTERIOSCLEROTIC VASCULAR DISEASE IN DIABETICS: RESULTS FROM LUMBAR SYMPATHECTOMY AND COMPARATIVE ANALYSIS WITH NONDIABETIC PATIENTS. A.M.A. Arch. Surg. *71*:460-67, September 1955.

The results from lumbar sympathectomy in 93 diabetic patients with symptomatic peripheral vascular disease have been presented and compared with results in 182 nondiabetic patients. In the absence of ulceration or gangrene, results in nondiabetics are somewhat better than in diabetic patients. After necrosis has developed, the results are essentially the same. Severity, duration, or adequacy of treatment of diabetes little affects the percentage of good results obtained. The amputation rate is higher in inadequately treated and in short-term diabetics. The nature of the vascular lesions and blood lipid changes and the relationship to the higher observed incidence of gangrene in diabetics have been discussed.

Breidahl, Harold D.; Priestley, James T.; and Rynearson, Edward H.: HYPERINSULINISM: SURGICAL ASPECTS AND RESULTS. Ann. Surg. *142*:698-708, October 1955.

The records of 91 cases in which the diagnosis of hyperinsulinism was made have been reviewed. In 48 cases, an adenoma of the islet cells was excised locally; in 26, partial pancreatectomy was performed; and in 3, total pancreatectomy was done. In the remaining 14 cases, various other procedures were carried out, including 6 in which only biopsy was performed. A tumor was encountered by the surgeon at the time of exploration in 64 of the 91 patients, and a tumor was subsequently demonstrated in 12 additional patients either by the pathologist or by the surgeon. Sixty-five per cent of patients were apparently cured, 24 per cent had had persistence of symptoms (including those with metastatic malignancy), 8 per cent died postoperatively, and 3 per cent were completely relieved for varying lengths of time (6 months to 11 years). In 15 patients, only normal pancreatic tissue could be demonstrated. One patient in the group reviewed is living and well more than 12 years after total pancreatectomy.

Brocq, P.; and Verne, J.: TENTATIVE CLASSIFICATION OF HYPOGLYCEMIAS. Presse méd. *86*:1770-74, 1954 (Abstr. in Prensa méd. argent. *42*:935, April 1, 1955).

According to Brocq and Verne, there is a ratio of 1/3 to 4 between the alpha and beta cells of the islets of Langerhans of the pancreas. So-called pancreatic hypoglycemia may be the result of (1) benign tumors, adenomas; (2) malignant tumors, carcinoma; (3) chronic pancreatitis; or (4) hyperplasia of the islets of Langerhans. In functional hypoglycemia, the pancreas is histologically normal. Symptoms of hypoglycemia vary in degree from the slight symptoms, such as hunger and epigastric discomfort with nervousness, to the mild neuropsychiatric disturbances and, finally, the most severe, hypoglycemic coma. A persistent fasting hypoglycemia is the most helpful laboratory finding for diagnosis. In functional hypoglycemia, the surgical approach by pancreatectomy has been advised as the only therapy in severe cases in which frequent hypoglycemic episodes are present. In eight cases so treated, a hyperplasia of the islet cells with predominance of beta cells over the alpha cells was found after histologic examination. (Spanish)

Clarke, D. W.: THE INFLUENCE OF POTASSIUM ION UPON GLUCOSE UPTAKE AND GLYCOGEN SYNTHESIS IN THE ISOLATED RAT DIAPHRAGM. Canad. J. Biochem. & Physiol. *33*:687-94, July 1955.

High concentrations of potassium ion in a buffering medium containing glucose cause reduced glucose uptake and glycogen synthesis in the isolated rat hemidiaphragm. Under similar conditions, but with glucose absent from the medium, glycogen breakdown remains unaffected, which indicates that the inhibitory effect of concentrated potassium is on a reaction or reactions involved in glucose uptake. The "insulin effect" on glucose uptake by isolated diaphragms from fed and fasted rats was unaffected by high potassium concentrations.

Collens, William S.; and Banowitch, Morris M.: INSULIN RESISTANCE: REPORT OF AN UNUSUAL CASE WITH REQUIREMENTS UP TO 7840 UNITS IN 24 HOURS. Metabolism *4*:355-63, July 1955.

A report is made of a diabetic with severe insulin resistance, who requires as much as 7,840 units of insulin in one day to control the diabetes.

Dogliotti, Giulio: PROGNOSIS OF DIABETES MELLITUS. Minerva med. *46*:1024-26, April 12, 1955 (Abstr. in Orientación méd. *4*:567-68, June 24, 1955).

Although the use of insulin has solved many of the problems concerning the prognosis in diabetes, the final outlook of these patients still remains uncertain because of the late appearance of degenerative complications. The pathogenesis of these complications has not been defined, and the only advisable recommendation for preventing them is to exert a close control of the diabetes. (Spanish)

Editorial: DIABETES MELLITUS—A SYMPTOM-COMPLEX. Connecticut M. J. *19*:821-22, October 1955.

Diabetes mellitus is an old story. The ancient Hindus called it the honey-urine disease; Avicenna, in the fifteenth century, noticed the sweet taste of the urine; and Matthew Dobson, in that never-to-be-forgotten year 1776, proved that the saccharine flavor was due to sugar. When, in 1889, von Mering and Minkowski demonstrated that ablation of the pancreas in animals led to the disease, it seemed as though the problem of its etiology had been fathomed. But "things are not always what they seem"; and later experience, both experimental and clinical, has shown that secretions of three other endocrine glands—the pituitary, the thyroid, and the adrenal—may cause or modify the typical diabetic picture. This may be defined as hyperglycemia and glycosuria with, at times, dehydration, ketonuria, and even diabetic coma. Inasmuch as all three glands are subject to diseases which may either increase or decrease certain hormones, it is only natural that abnormalities in these secretions may modify diabetes either favorably or unfavorably. Diabetes mellitus should not be regarded as a specific disease entity with a fixed and unalterable etiology but rather as a symptom complex which may result from a variety of causes. A similar situation has been shown to prevail in some other conditions originally regarded as specific diseases (cirrhosis of the liver, for example) and in all likelihood will, with increased study and experience, be shown to be true of other so-called diseases.

Edwards, Margaret H.; Pessel, Johannes F.; Rathmell, Thomas K.; and Wise, John S.: HYPERINSULINISM AND PREMENSTRUAL TENSION: REPORT OF A CASE OF HYPERPLASIA OF THE ISLETS OF LANGERHANS. Am. J. Obst. & Gynec. *70*:1129-32, November 1955.

A case is presented which illustrates marked aggravation of hypoglycemia in the premenstrual period by a functioning hyperplasia of the islets of Langerhans. Oral glucose tolerance curves before and after the onset of menses were totally dissimilar; surgical resection of two-thirds of the pancreas resulted in a reversal of symptoms and a temporary mild diabetic state. An estrogen-progesterone imbalance is thought to be a factor in premenstrual hypoglycemia; the mechanism whereby a relative excess of estrogens may depress the blood sugar level is briefly discussed.

Eger, Alban W.; and Putzki, Paul S.: SPONTANEOUS HYPOGLYCEMIA DUE TO ISLET CELL TUMORS OF THE PANCREAS: REPORT OF A CASE DUE TO MULTIPLE ADENOMAS. M. Ann. District of Columbia *24*:463-68, September 1955.

Removal of two adenomas from the pancreas was curative of serious symptoms due to hypoglycemia. Invasion of the nodular capsule and of the pancreas beyond the capsule was noted on microscopic examination and should suggest a diagnosis of malignancy with a poor prognosis. However, previously reported similar cases were not associated with a malignant course of the illness.

Evans, R. Winston; Littler, T. R.; and Pemberton, H. S.: GLYCOGEN STORAGE IN THE LIVER IN DIABETES MELLITUS. J. Clin. Path. *8*:110-13, May 1955.

In a diabetic clinic of 800 patients, a new syndrome has been described in four cases with a brittle type of diabetes mellitus, associated with enlargement of the liver due to glycogen storage and recurrent attacks of hypoglycemia. The cases are described from both clinical and biochemical aspects. The nature of these findings is discussed with particular reference to von Gierke's disease, the use of "glucagon" (HGF), and the more common syndrome of diabetes mellitus.

Fanconi, G.: THE SO-CALLED FREE DIET (BETTER NORMAL DIET) IN THE TREATMENT OF INFANTILE DIABETES. Schweiz. med. Wchnschr. *85*:75-77, Jan. 22, 1955.

From 1923-1932, Fanconi treated his patients with a low-carbohydrate diet (30 grams of carbohydrate per day); from 1932-1946, with his "low-protein fruit-vegetable diet." He discovered that 16 years after the beginning of diabetes, all his patients had developed nephropathy; and after 21 years' duration, all the patients had died. Therefore, in 1946, he started to use free diet according to Stolte-Lichtenstein, which he modified slightly by warning the children to avoid overnutrition and sugar and fine starches. After 8 years' experience, Fanconi declares free diet to be "the treatment of choice" for infantile and juvenile diabetes. (German)

Gaarenstroom, J. H.; and Rümke, Chr. L.: CHANGES IN THE BLOOD SUGAR LEVEL DURING THE FIRST HALF HOUR AFTER ALLOXAN INJECTION AND THE INFLUENCE OF SEX ON THESE CHANGES. Acta endocrinol. *19*: 333-39, August 1955.

The authors have previously described the results of a study on the blood sugar levels 30, 60, and 150 minutes after intravenous administration of alloxan in normal rats. One result included an initial slight fall preceding the usual rise in the blood sugar levels in animals which became diabetic, in contrast to the immediate rise in those which did not develop diabetes.

Further review of their data suggests a difference between the reactions in the male and female rats used. Thirty minutes after alloxan, the blood sugar level in

the male rats which subsequently became diabetic was found to be higher than in the females. The same difference between the blood sugar values of rats of both sexes was observed in those which did not develop diabetes.

This study was designed to extend and to try and confirm the apparent sex differences observed in earlier studies. After small changes in the experimental procedures, the earlier apparent differences were no longer found.

Gibbs, Gordon E.: A Study in Outpatient Management of Juvenile Diabetes. Nebraska M. J. 40:399-402, November 1955.

Close control of juvenile diabetes is important as a means of retarding degenerative changes leading to blindness, renal failure, etc. A simple system of outpatient management based on flexible insulin dosage, one daily insulin injection, and some fitting of the diet to diurnal insulin-action pattern produced a degree of control which conformed to relatively high standards.

Goodman, Joseph I.: Clinical Discussion: Management of Uncomplicated Diabetes Mellitus. Metabolism 4:446-458, September 1955.

A simple and reasonably accurate method for treatment of the diabetic patient, which takes a minimum of effort, is presented.

Goodman, Joseph I.: The Use of a Light Cradle in the Treatment of Diabetic Ulcers, Gangrene and Decubitis Ulcers. Am. J. Surg. 90:676-79, October 1955.

As a result of the observations of various investigators, it is known that marked peripheral vasoconstriction is present in the toes and fingers, as indicated by low skin temperatures, at environmental temperatures of 17° to 20°C. (63.5° to 68°F.). On the other hand, under environmental temperatures of 31° to 33°C. (88° to 91.5°F.), marked vasodilatation exists in the fingers and toes, as evidenced by skin temperatures approximating the readings on the forehead and trunk. It has been shown that blood flow through the extremities can be increased by raising the environmental temperature. In a thermostatically controlled room, fluctuations in the skin temperature readings closely parallel the elevation or fall of the room temperature. For each degree rise or fall of the room temperature, there is a corresponding change in the skin temperature. There are, however, two critical temperatures, approximately 80° and 89°F., at which this pattern does not hold. At 80°F., there is a sudden increase in the skin temperature readings of the hands of 7 to 8 degrees; and at 89°F., a similar occurrence takes place in the feet. Above these two critical temperatures, the parallelism of the room and skin temperatures of the extremities is maintained. The sudden rise in the skin temperature of the hands and feet, at 80°, and 89°F. respectively, reflects the release of vasomotor tone in the arterioles. Thermoregulation of the body is maintained by a vasomotor mechanism which controls the volume of blood flow to the cutaneous capillaries of the hands and feet. For example, at excessive environment temperatures blood is brought to the skin surface by the inhibition of vasomotor (sympathetic) tone, so that cooling is facilitated. In employing the light cradle, advantage is taken of the physiologic principle of the effect of environmental temperature on peripheral blood flow; so that, at temperatures of 90° to 95°F., a maximum blood flow in the extremities is assured throughout the entire twenty-four-hour period. The light cradle employed here must not be confused with a baker or other apparatus producing high temperatures, with the attendant danger of burns and other untoward effects. Under a light cradle, where the temperature ranges from 90° to 95°F., there is no danger of burns whatsoever.

Goto, Yoshio: Significance of the Two-Dose Glucose Tolerance Test. Metabolism 4:323-32, July 1955.

One-dose tolerance tests and two-dose tolerance tests were performed in the same subjects. The assumed two-dose tolerance curve was drawn from the one-dose tolerance curve by calculation, and this assumed two-dose tolerance curve was compared with the actual two-dose tolerance curve. The results show that the calculated curves correspond closely with the measured tolerance curves. From these results, the Staub effect seems to have no significance.

Hayes, Daniel W.: Diabetes Mellitus, its Types and Treatment. Clin. Med. 2:687-90, July 1955.

Awareness of the "potential" diabetic, the "prediabetic," and the "actual" diabetic patients in the physician's practice is necessary to the capable practice of medicine or any of its specialties. Once discovered, watchful waiting in the case of the potential and prediabetic states, with removal of predisposing factors such as obesity, associated diseases, and other stresses, is the proper course. Accurate and constant care of the "actual" diabetic, with proper diet and exercise and the use of insulin, if needed, makes the diabetic indeed a healthy and useful citizen.

Henneman, Dorothy H.; Altschule, Mark D.; and Goncz, Rose Marie: Effect of Intravenous Administration of Glutathione in Man on Blood Glucose, Some Other Blood Carbohydrates and Serum In-

ORGANIC PHOSPHATE. Metabolism 4:433-45, September 1955.

Glutathione was infused intravenously in thirty-one psychotic patients and three normal men in forty-nine experiments. No pyrogenic or toxic effects were observed when doses as high as 0.5 gm. per kg. of body weight were dissolved in isotonic sodium chloride solution and infused slowly over a two-hour period. Given alone to fasting subjects, glutathione caused slight and probably insignificant decreases in blood glucose and lactic and pyruvic acids; citric acid concentrations fell. There were striking decreases in the concentration of serum inorganic phosphate. When given prior to glucose administration, it did not affect glucose tolerance but did decrease the elevation in blood lactic acid concentration observed after the administration of glucose alone. It increased the fall in serum inorganic phosphate observed after the intramuscular injection of epinephrine and after the administration of ACTH and glucose together. However, it decreased the glucose-induced phosphate fall somewhat. Inhibition of the action of ACTH and hydrocortisone was not observed; on the contrary, exaggeration of some of the metabolic changes induced by ACTH or hydrocortisone occurred in most instances. In man, the action of glutathione appears to be related to phosphorylating mechanisms, as reflected by changes in serum inorganic phosphate concentration.

Herold, A. A., Sr.: SIGNIFICANCE OF OVERWEIGHT IN ETIOLOGY, PROGNOSIS, AND TREATMENT OF DIABETES MELLITUS, WITH REPORT OF CASES. J. Louisiana M. Soc. 107:403-06, October 1955.

Since metabolism of fats without presence of carbohydrates produces ketone bodies, we should be careful not to eliminate carbohydrates entirely from dietary menus. Never neglect the liver's need for carbohydrates; for, with exhaustion of glycogen, this organ may throw out toxic substances, which develop into ketone bodies. Insulin not only metabolizes sugar but also enables the liver to store it as glycogen. Practically all slender diabetics, like growing children, require insulin with proper control of calories but with liberal carbohydrates. Finally, obesity is the main thing to avoid in those with diabetic tendency or diabetic family history, and the principal weapon with which to combat it is caloric restriction.

Hilgartner, Henry L.: DIABETIC RETINOPATHIES: EFFECT OF LIPOTROPIC SUBSTANCES (LIPOTRIAD) IN TREATMENT. Texas J. Med. 51:735-37, November 1955.

The lipotropic substances choline, inositol, and methionine have no apparent effect on the diabetic per se, such as reducing the blood sugar or the insulin and diet requirements. The lipotropic substances do not tend to produce retinal hemorrhages, since these are a normal diabetic characteristic. The lipotropic substances apparently help in the absorption of the retinal hemorrhages and exudates. The lipotropic substances seem definitely to improve the feeling of well-being in the patients. The older diabetic patient follows a more regular and favorable course on the lipotropic therapy than does the juvenile group.

Kadota, Ichiro; and Kurita, Mitsutaro: HYPERGLYCEMIA AND ISLET CELL DAMAGE CAUSED BY NICKELOUS CHLORIDE. Metabolism 4:337-42, July 1955.

After the administration of 10 mg. per kg. of nickelous chloride in rabbits, we observed a transitory hyperglycemia in about half the animals. All rabbits administered 15 to 20 mg. per kg. of nickelous chloride showed hyperglycemia; they reached maximal levels in one to two hours and returned to normal after 24 hours. In the islets of these rabbits, we found destruction of alpha cells and, to a lesser degree, beta-cell damage with degranulation. These results are similar to those produced with cobaltous chloride; however, the cobalt hyperglycemia was less marked.

Klein, R.; Weigand, F. A.; Iunes, M.; and Greenman, L.: CORTICOIDS IN SERUM OF CHILDREN WITH TREATED DIABETES MELLITUS. Pediatrics 17:214-20, February 1956.

Free serum corticoids in children with treated diabetes mellitus were found to be significantly higher than those in normal children. The level of serum corticoids was directly related to the amount of reducing substances found in the urine and was inversely related to the carbon dioxide content of the sera.

Kolb, Felix O.; de Lalla, Oliver F.; and Gofman, John W.: THE HYPERLIPEMIAS IN DISORDERS OF CARBOHYDRATE METABOLISM: SERIAL LIPOPROTEIN STUDIES IN DIABETIC ACIDOSIS WITH XANTHOMATOSIS AND IN GLYCOGEN STORAGE DISEASE. Metabolism 4:310-17, July 1955.

Serial studies of diabetic acidosis indicate that the lipoprotein transport derangement is ameliorated with reestablishment of diabetic control and becomes marked again even with moderate diabetic "decontrol" and acidosis. The dynamics of alteration of low and high-density lipoprotein transport in the course of therapy of diabetic acidosis is described in detail and correlated with the clinical picture. A basic similarity between diabetic xanthomatosis and chronic xanthoma tuberosum is suggested. Enhanced atherogenesis would be anticipated during episodes of diabetic "decontrol" associated with

acidosis. The similarity in the lipoprotein transport derangement of diabetic acidosis and glycogen storage disease suggests that hyperglycemia per se is not the likely underlying factor but rather that a defect in carbohydrate utilization may be such a factor. That a defect in carbohydrate metabolism may exist in "essential hyperlipemia" as well seems worth-while investigating.

Kvamme, Elling: BIOCHEMICAL ASPECTS OF DIABETES. Nord. med. *54*:1409-12, Sept. 15, 1955.

The article is a review of recent important findings in experimental diabetes. (Norwegian)

Lambie, A. T.; and MacFarlane, A.: A CLINICO-PATHOLOGICAL STUDY OF DIABETIC GLOMERULOSCLEROSIS. Quart. J. Med. *24*:125-42, April 1955.

A clinico-pathological study of diabetic glomerulosclerosis was made in 120 consecutive cases of diabetes mellitus coming to post-mortem examination within a three-year period. The lesion was found in 46 per cent of cases and was slightly more common in women than in men. Diabetic glomerulosclerosis is considerably more common in poorly controlled diabetes. Poor control is, therefore, one factor in the production of the lesion. The average duration of diabetes in patients with glomerulosclerosis was 11.7 years; in those without the lesion, 7.3 years. The duration of diabetes is an important causal factor in the production of the lesion, particularly in the younger age group. The severity of glomerulosclerosis is related to the duration of the diabetes. Most cases of glomerulosclerosis are found in the seventh and eighth decades; but the majority of cases of diabetes, with or without renal lesions, occur in these age groups. In most decades, however, the incidence of the lesion is approximately 50 per cent. The incidence of glomerulosclerosis does not bear any relation to the severity of the diabetes. Massive albuminuria appears to be related to the presence of exudative lesions in the glomeruli. Most patients with mild glomerulosclerosis do not show clinical evidence of its presence, and seven cases of severe glomerulosclerosis were not clinically obvious. Young diabetic patients with glomerulosclerosis show clinical evidence of the lesion more commonly than do elderly diabetics.

Langsjoen, George E.: THE ETIOLOGY OF DIABETIC NEUROPATHY. J. Kansas M. Soc. *56*:582-94, October 1955.

It is evident that arteriosclerosis cannot account for the picture of diabetic neuropathy in all patients. If diabetic neuropathy were limited to the older age group, this would be an important factor. One might state that arteriosclerotic changes in nutrient arteries may play an aggravating role in older diabetics and in those refractory to diabetic management. More pathologic studies would be helpful. In consideration of vitamin deficiency, some writers maintained that they obtained complete cures; others found no relief with thiamine administration. The question arises as to whether or not the latter were dealing with true diabetic neuropathies. If dietary intake, absorption, utilization, and excretion factors could be accurately determined, this faction would achieve more significance. It is generally agreed that diabetic neuropathy usually occurs after prolonged periods of diabetic neglect. DeJong's interesting theory that excessive oxidation of fats leads to demyelization of peripheral nerves bears further investigation. At least this is a theory which gives a clearer picture than one which states that diabetic neuropathy is due to some vague upset in carbohydrate metabolism. No conclusion was reached concerning Rundle's idea that inability to oxidize carbohydrate, which is the principal foodstuff of nerves, was a possible etiological factor. Levitt's hypothesis concerning diabetic neuropathy's being precipitated by diabetic management appears the most attractive. However, in these cases, it would be necessary to obtain relief of symptoms by thiamine administration, which is not always possible.

LeCompte, Philip M.: VASCULAR LESIONS IN DIABETES MELLITUS. J. Chronic Dis. *2*:178-219, August 1955.

Diabetes mellitus is associated with a high incidence of vascular disease involving large and small arteries, capillaries, and venules. Disease of the large arteries (atherosclerosis, medial calcification, intimal fibrosis) in diabetes does not seem to differ in kind from that seen in nondiabetics but is often more severe in diabetics. This is especially striking in females. Disease of the capillaries, especially those of the kidney and the eye (perhaps because of higher pressure in these organs), with deposits of a hyaline, polysaccharide-containing substance, is so characteristic of diabetes as to be almost pathognomonic. Modern technics suggest that the deposits have a distinctive physicochemical constitution. The vascular lesions of diabetes mellitus have not been reproduced unequivocally in experimental animals, in the sense of having been induced by the diabetic state alone. Those lesions that have been described, especially in the kidney, cannot be regarded as an exact duplicate of the human lesion. Something like the "exudative lesion" in the kidney, and perhaps retinal microaneurysms as well, can be produced in rabbits with large doses of cortisone. Vascular lesions in diabetes mellitus are accompanied sometimes, but not always, by elevations of the serum levels of cholesterol, lipoproteins, and esterified fatty acids and also (especially the late capillary lesions) by elevated serum polysaccharides. Whether any of these have a causal relation to

the vascular lesions is uncertain. It is conceivable that a fundamental disorder of carbohydrate metabolism in diabetes may affect the polysaccharide-containing ground substance of blood vessels of various sizes in such a way as to predispose to the lesions. It is uncertain whether the vascular disturbances are related to the diabetes genetically or by way of some other common factor, i.e., as concomitants, or causally, i.e., as complications. The fact that the vascular lesions tend to increase in severity with duration of the diabetes suggests that the second hypothesis may be true; and on this basis, careful control of the diabetes may tend to prevent the lesions.

Mallet, Robert: THE DIFFERENT INSULINS AND THEIR USE IN CHILDHOOD. Courrier 5:290-91, May 1955.

The aims of insulin therapy are the same whether a diabetic child receives a strictly weighed diet or not. A diabetic child may be regarded as under adequate control if his physical development continues normally, if he is not very thirsty, if his daily sugar excretion is not higher than 10 to 20 gm., if his liver is not enlarged, and if he does not have insulin reactions. This last condition is the hardest to fulfill. Under present conditions, the risk of hypoglycemia in children is greater than the risk of ketosis. Whatever diet is chosen, it is essential that an adequate carbohydrate ration is spread regularly over the 24 hours. In order to treat a diabetic child adequately, very strict controls must be enforced. Two, three, or even four times a day, tests for urinary ketosis should be made and the glucose excretion measured. The daily dose of insulin will depend on the urinary findings. Occurrence of ketosis, abnormal thirst, or too high a glucose excretion requires a higher dosage. Even mild hypoglycemia should command a reduction of the dosage. As regards the selection of the appropriate variety of insulin, it will be made according to the child's individual response. A choice which has proved excellent in one patient may be unsatisfactory in another, and a certain empiricism may be necessary. Except for cases with impending ketosis, ordinary insulin is now seldom used alone. Its use, hitherto justified by the danger of protracted hypoglycemia due to protamine zinc insulin, has the great disadvantage of requiring several daily injections. (English)

Peden, J. C., Jr.; Riley, J. S.; Bond, L.; and Elman, R.: BLOOD SUGAR LEVELS FOLLOWING INTRAVENOUS INFUSION OF GLUCOSE AND FRUCTOSE IN ADULTS. Metabolism 4:318-22, July 1955.

Blood sugar (total reducing) levels were observed during and for three hours after the intravenous administration of 10 per cent glucose and fructose, given on alternate days at a uniform rate to each of 12 essentially normal hospitalized subjects. The blood sugar levels immediately after the infusion of glucose were much higher than after fructose; whereas, during the postinfusion period, the difference was reversed. Minimal blood sugar levels after fructose were essentially the same as the fasting levels; the average minimal postinfusion level with glucose was about 19.3 mg. per cent lower. Clinical manifestations of hypoglycemia were seen in one subject who received glucose and in none who received fructose. The clinical implications for the use of fructose to combat postinfusion and postabsorption hypoglycemia are discussed.

Reeves, Robert L.; Steenrod, William J.; Crampton, Joseph H.; and Palmer, Lester J.: LENTE INSULIN. A CLINICAL TRIAL. Northwest Med. 54:1090-94, October 1955.

Clinical comparison of NPH and Lente insulins in a small number of "concave reactors" and "convex reactors" suggests that the former can be better controlled on a single daily dose of Lente insulin, whereas the latter are better controlled on a mixture of NPH and crystalline insulins.

Solez, Chester: PREMATURE VASCULAR DEGENERATION IN DIABETES MELLITUS: PATHOGENESIS, AND RELATION TO AGING. J. Am. Geriatrics Soc. 3:804-10, October 1955.

Arteriosclerosis is probably a structural result of a physiologic disorder. There is growing evidence that hormones may be one of a wide variety of factors related to the pathogenesis of arteriosclerosis. The premature vascular degeneration observed in association with insufficient insulin therapy in diabetes mellitus affords another opportunity for gaining insight. There may be some common physiologic denominator for the arterial degeneration occurring in Cushing's syndrome, "insulin-deficient" diabetes, and aging. Premature arteriosclerosis occurs in both diabetes due to hyperadrenocorticism and in "insulin-deficient" diabetes. The action of glucocorticoids from the adrenal cortex is protein-antianabolic, or catabolic. Excess glucocorticoids (as in Cushing's syndrome) cause a wasting of protein tissue throughout the body. The protein tissue of blood-vessel walls is probably involved too. Fatty replacement, calcification, and sclerosis may follow in the wake of degeneration of protein tissue in the vessel walls. Insufficient insulin also causes antianabolic, or catabolic, derangements affecting the protein tissue of the body. Here, the protein tissue in the blood-vessel walls may suffer and be replaced by tissue of a different character. This may give rise prematurely to anatomic changes of senescent degeneration—i.e., arteriosclerosis. Aging itself is a type of protein catabolism, or a decreasing protein anabolism; therefore,

it is not surprising that arteriosclerosis occurs prematurely in the protein-catabolic environment of diabetes. Protein antianabolism, or catabolism, is not the only mechanism of production of arteriosclerosis in diabetes. However, this aspect of the relationship of diabetes to premature arteriosclerosis should receive more attention. This concept may help to explain why giving optimum amounts of insulin to diabetic patients prevents or delays the onset of vascular degeneration.

Spiess, H.: LONG-ACTING INSULINS FOR THE TREATMENT OF JUVENILE DIABETES MELLITUS. Deutsche med. Wchnschr. 80:1170-72, Aug. 19, 1955. (English summary on page 1210.)

Since the summer of 1953, 21 children 5 to 13 years of age have been treated with "Lente" and "Long" insulin. Local reactions were absent, on the whole, although in the beginning some of the children complained of a transient burning sensation. However, some reactions at the site of injection, viz., hyperplasia and lipodystrophy, also have to be reckoned with in this type of insulin. If "Lente" or "Long" insulin is given in the morning, maximal effects are reached in the early afternoon. The treatment aims at reducing the urinary excretion of sugar to below 20 gm. per day and at keeping the level of the blood sugar below 200 mg. p.c. After having been adjusted to "Lente" insulin (an average of 20-40 units per day, occasionally more), 7 out of 11 children could be maintained in a fair "carbohydrate balance." Carbohydrate metabolism was satisfactorily balanced in 5 of the 10 children receiving "Long" insulin but was unsatisfactory in 2. Hypoglycemic reactions seemed to occur more rarely than with the older types of insulin. (German)

Trethowan, William H.: PERSISTENT SENSORY SYMPTOMS FOLLOWING SPONTANEOUS HYPOGLYCEMIA: RESPONSE TO CHLORPROMAZINE. REPORT OF A CASE. J. Nerv. & Ment. Dis. *121*:274-78, March 1955.

A patient suffering from persistent neuritic symptoms following severe spontaneous hypoglycemia is described. Although the exact basis cannot be determined, it is thought that the symptoms may be the result of irreversible neuritic changes either due to sudden relative vitamin B_1 deficiency associated with the abnormality of carbohydrate metabolism or the result of some other direct or indirect action of insulin. Comment is made on the alleviation of the patient's symptoms by Chlorpromazine.

Vartiainen, Ilmari; and Telkkä, Antti: EFFECT OF HIGH FAT DIET ON THE PANCREATIC ISLETS OF RAT. Ann. med. int. Fenniae *44*:79-88, 1955.

Five rats were kept for a year on a high-carbohydrate diet (carbohydrate, 80 per cent; protein, 15 per cent; fat, 5 per cent of total calories). The composition of the diet was based on the selections made by healthy rats in several investigations. This group served as a control series. Another group was kept in the same conditions on a high-fat diet (carbohydrate, 5 per cent; protein, 15 per cent; fat, 80 per cent of total calories). In blood sugar values, glucose tolerance, and need for water, the high-fat group did not significantly differ from the control group. Necropsy revealed a lower liver weight in the high-fat group but no cirrhotic changes. The weight of adrenal glands was higher, due to hypertrophy of the cortex. The most noticeable change was the diminution of Langerhans' islets in the high-fat group (0.9 per cent of the pancreatic tissue) in comparison with the islets of the high-carbohydrate group (2.3 per cent).

Vartiainen, Ilmari; and Telkkä, Antti: EXPERIMENTS IN FEEDING NORMAL RATS ON DIETS SELECTED BY DIABETIC RATS. Ann. med. int. Fenniae *44*:165-70, 1955.

Five groups of rats, of five each, were fed for sixteen months with diets selected in earlier experiments by normal and alloxan-diabetic rats. Histological examination of the pancreas, liver, kidneys, adrenals, thyroids, and crystalline lenses revealed no alterations between the groups. Another diet, containing 80 per cent lactose and given for seven months, produced a cataract in all the rats of another group. In the histochemical demonstration of the sulfhydryls, the cataractous area remained practically unstained.

Waagstein, P.H.D.: HYPERLIPEMIA AND HYPERGLYCEMIA IN DIABETES MELLITUS. DEMONSTRATION OF ARTERIOVENOUS LIPOID DIFFERENCE. Scandinav. J. Clin. & Lab. Invest. 7:15-21, 1955.

Fifty hospitalized diabetics have been followed, with determination of the total lipid content of the blood besides determination of the fasting blood sugar. The two curves were found to run parallel. Two cases of pronounced hyperlipemia showed a course quite independent of that of hyperglycemia. When the patient was confined to bed, the rise in hyperlipemia appeared suggestive of mechanical factors. Fever was found to lower the level of fasting lipemia. Thirty fat-tolerance tests, with and without administration of insulin, illustrated the influence of insulin upon the course of alimentary hyperlipemia. Accidentally, a constant arteriovenous lipid difference was ascertained on determination of the total lipoid content on blood obtained from the ear and on venous blood.

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COMMITTEE SCHEDULE SIXTEENTH ANNUAL MEETING

Committees and other organizational groups of the American Diabetes Association are scheduled to meet at the following times during the period of the Sixteenth Annual Meeting to be held June 9-10 in Chicago. All meetings are at The Drake, the headquarters hotel. Committee members will be notified if it should be necessary to make any changes in this schedule.

Executive Committee: June 5, from 10 a.m.; June 6, 9 a.m.—12 m. *Committee on Policies*: June 6, from 1 p.m. *Nominating Committee*: June 7, 8 a.m.—10 a.m. *Committee on Information for Diabetics*: June 7, 8 a.m.—10 a.m. *Committee on Camps*: June 7, 10:15 a.m.—12:30 p.m. *Committee on Membership*: June 7, 10:15 a.m.—12:30 p.m. *Steering Committee of the Committee on Detection and Education*: June 7, 10:15 a.m.—12:30 p.m. *Committee on Affiliate Associations*: June 7, 12:45 p.m.—2:15 p.m. *Committee on Scientific Awards*: June 7, 12:45 p.m.—2:15 p.m. *Committee on Employment*: June 7, 2:30 p.m.—4:30 p.m. *Committee on Professional Education*: June 7, 2:30 p.m.—5:30 p.m. *Subcommittee on Standardization of the Committee on Professional Education*: June 7, 5:30 p.m.—6:30 p.m. *Committee on Food and Nutrition*: June 7, 8 p.m.—10 p.m. *Committee on Scientific Publications*: June 7, 8 p.m.—10 p.m. *Committee on Scientific Exhibits*: June 8, 8 a.m.—9:15 a.m. *Committee on Scientific Programs*: June 8, 8 a.m.—9:15 a.m. *Council*: June 8, 9:30 a.m.—12:30 p.m., 2 p.m.—5:30 p.m. *Subcommittee on Advertising of the Committee on Scientific Publications*: June 8, 12:30 p.m.—2 p.m. *Council with Board of Governors*: June 8, 6 p.m.; 7:30 p.m.—10 p.m. *Board of Governors*: June 9, 1 p.m.—2:30 p.m. *Assembly of Delegates*: June 9, 2:30 p.m.—5:30 p.m. *Editorial Board,* DIABETES: June 10, 12 m.—2:00 p.m. *Committee on Detection and Education*: June 11, 9:30 a.m.—12 m., (open meeting).

DATE CHANGE FOR FIFTH POSTGRADUATE COURSE

The Fifth Postgraduate Course in Diabetes and Basic Metabolic Problems will be held Wednesday through Friday, Jan. 30—Feb. 1, 1957, at the Student Union of Ohio State University, Columbus, Ohio, instead of Jan. 23-25 as previously announced. The Course will be sponsored by the American Diabetes Association in cooperation with the College of Medicine of the University. George J. Hamwi, M.D., Columbus, will be Director, and Thomas P. Sharkey, M.D., Dayton, Associate Director.

MEAL PLANNING PUBLICATIONS AVAILABLE

"Meal Planning with Exchange Lists," a booklet prepared to help diabetics select foods for their meals, Meal Plans No. 1 through No. 6, and a Diabetic Diet Card for Physicians, may now be obtained through the offices of the American Diabetes Association, 1 East 45th St., New York 17, N. Y. Copies are also available from The American Dietetic Association.

A new price schedule for this material was effective May 31. Single copies of the booklet "Meal Planning with Exchange Lists" are available at $.15 each; 100 at $6.50; 1,000 at $50.00. The separate Meal Plans (1-6), designed for use with "Meal Planning with Exchange Lists," may be obtained at $.05 per meal plan; 100 copies of one or an assortment at $2.00; 1,000 copies of one or an assortment at $18.00. The Diabetic Diet Card for Physicians, containing information about exchange lists and food values, is available at $.05 per card; $2.00 per 100 and $18.00 per 1,000.

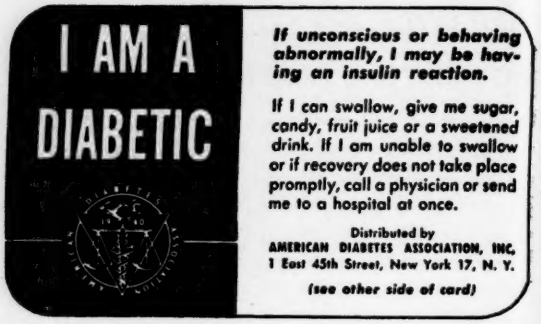
Meal Plan No. 1 (carbohydrate 125 grams, protein 60 grams, fat 50 grams) contains 1200 calories; Meal Plan No. 2 (carbohydrate 150 grams, protein 70 grams, fat 70 grams) 1500 calories; Meal Plan No. 3 (carbohydrate 180 grams, protein 80 grams, fat 80 grams) 1800 calories; Meal Plan No. 4 (carbohydrate 220 grams, protein 90 grams, fat 100 grams) 2200 calories; Meal Plan No. 5 (carbohydrate 180 grams, protein 80 grams, fat 80 grams) 1800 calories; Meal Plan No. 6 (carbohydrate 250 grams, protein 100 grams, fat 130 grams) 2600 calories.

This material was prepared by Committees of the American Diabetes Association and The American Dietetic Association in cooperation with the U. S. Public Health Service. It was formerly distributed by the Health Publications Institute of Raleigh, North Carolina.

Order forms are available on request.

ADA IDENTIFICATION CARD

An identification card for diabetics, issued by the American Diabetes Association and pictured on this page, is now available. The card will fit the average pocket or purse wallet, and bears the official seal of the Association. It was developed by the Committee on Information for Diabetics and plans for distribution were completed at the 1955-56 Interim Council Meeting. The price is $.10 each in quantities of one through nine and $.05 each in quantities of ten or more, both prices including handling and shipping. Upon recommendation of the Committee, the Council has authorized sending a sample card in the near future to each Active Member ot the Association.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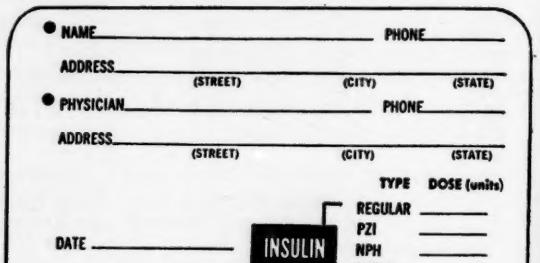
(see other side of card)

FRONT



BACK

LILLY AWARD ESTABLISHED

An award consisting of $500 and a gold medal and known as the Lilly Award, has been established. The Council of the American Diabetes Association accepted the recommendation of the Committee on Scientific Publications regarding its establishment at the 1954-55 Interim Meeting held in Philadelphia Jan. 22-23, 1955. The annual award will be supported by Eli Lilly and Company.

The following stipulations govern the contest for the Award.

Purpose: To recognize demonstrated research in the field of diabetes, taking into consideration independence of thought and originality.

Eligibility: Any investigator in an appropriate field of work closely related to diabetes who is less than forty years of age on January 1 of the year in which the award is made. The research will not necessarily be judged in comparison to the work of more mature and experienced workers. The candidate should be a resident of the United States or Canada.

Nominations: Nominations for the award will be solicited from the members of the American Diabetes Association. Such nominations will be requested by re-

peated notices to be published in DIABETES. Names of nominees will be sent to the Chairman of the Committee on Scientific Awards and must be received before January 1 of the year of the award. The nomination should be accompanied by full information concerning the nominee's personality, training, and research work. Six copies of each item should be submitted. No member may send in more than one nomination. A list of the nominee's publications, if any, and six copies of the publication or manuscript for which the award is to be given should also accompany the nomination. The nominee should be actively engaged at that time in the line of research for which the award is to be made.

Announcement: The name of the winner will be announced in the program of the Annual Meeting of the Association, and the award presented at that meeting. The winner will, subject to the approval of the Committee on Scientific Programs, be invited to present a paper on the subject of his work. Papers considered for the award must be submitted with the idea that they will be published in whole or in part in DIABETES if found acceptable to the Editor and/or the Editorial Board. If the Committee should decide that no outstanding work has been presented for this consideration, the award will not be made.

Award: In addition to the monetary award and the gold medal, traveling expenses will be given to make it possible for the recipient to receive his award in person at the Annual Meeting.

AVAILABILITY OF RESEARCH FELLOWSHIP

The Committee on Research and Fellowships of the American Diabetes Association plans to award at least one Fellowship for the academic year 1957-58. Requests for application forms and other inquiries should be addressed to the Association's Executive Director, Mr. J. Richard Connelly, who will forward the information to the Committee.

COMMITTEE CHAIRMANSHIP

Louis K. Alpert, M.D., has accepted appointment as Chairman of the Committee on Detection and Education for 1956. Activities of this important Committee reach their peak during Diabetes Week, which will be observed from November 11 to November 17 this year.

NEW MEMBERS

Active

The following were elected as of May 1, 1956:

Alabama	
Crow, Charles B.	Birmingham
California	
Ogren, Roy S.	Redwood City
Wynekoop, Waldo W.	Bellflower
Florida	
Cone, Adolph B.	Jacksonville Beach
Illinois	
Dolkart, Ralph E.	Chicago
Indiana	
Jones, Roland W.	Indianapolis
Kansas	
McClintock, Edward A.	Topeka
Kentucky	
Urbach, Stuart	Louisville
Michigan	
McCain, French H.	Birmingham
Minnesota	
Burgerman, Arthur	Rochester
Law, William McConnell	Rochester
Missouri	
Hartroft, W. Stanley	St. Louis
New Hampshire	
Walsh, William C.	New Hampton
New York	
Deane, Norman	New York
Kircher, Raymond F.	Albany
Rosenberg, Benjamin A.	Brooklyn
Ohio	
Pritz, Walter L.	Cincinnati
Oklahoma	
Taylor, John R.	Kingfisher
Pennsylvania	
LeWinn, Edward B.	Philadelphia
Robinson, Frederick A.	Philadelphia
Sullivan, Jere J.	Lancaster
Vincent, Lyle D.	Philadelphia
Texas	
Allen, John B.	Dallas
Baxter, M. Ruth	Galveston
Bush, William Leslie	Dallas
Culmer, Harold H.	Dallas
Eckhardt, James W.	Austin
Ewing, Stanley F.	Sinton
Hamilton, Con D., Jr.	Cleburne
Holland, Lang F.	Austin
Kinard, Conrad L.	Denton
Martin, Thomas A., Jr.	Dallas
Mason, Morton F.	Dallas
Merrick, Benjamin A.	Dallas
Ramey, Lindell E.	Kingsville
Reynolds, William S., Jr.	Dallas
Roosth, Harold	Tyler
Ross, Nealie E., Jr.	Ft. Worth
Sanders, O. Perdue	Dallas
Smith, Travis	Abilene
Tunnell, John W.	Taft
Voss, Alpheus H.	Odem
Washington	
Narahara, Hiromichi T.	Seattle
Wisconsin	
Olson, Carroll R.	Milwaukee

Other Countries

Argentina	
Cabarrou, Arturo	Buenos Aires
Caino, Hector V.	Buenos Aires
Canada	
Brown, Gordon D.	Alberta
Italy	
Faelli, Carlo N.	Rome
Mexico	
Peschard, Armando	Mexico, D.F.

NEWS OF AFFILIATE ASSOCIATIONS

THE CHICAGO DIABETES ASSOCIATION will sponsor a Public Meeting at 8:15 p.m., Thursday, June 7, at the John B. Murphy Memorial Auditorium, 50 East Erie Street, Chicago, prior to the Sixteenth Annual Meeting of the American Diabetes Association June 9-10. The program includes a Panel Discussion by the following guest speakers, all of whom are Councilors of the American Diabetes Association: Charles H. Best, M.D., Toronto, "Past, Present and Future of Diabetes"; Francis D. W. Lukens, M.D., Philadelphia, "Oral Insulin Substitutes"; Alexander Marble, M.D., Boston, "Complications of Diabetes"; Blair Holcomb, M.D., Portland, Oregon, "Living with Diabetes."

THE NEW YORK DIABETES ASSOCIATION (Clinical Society) held an open meeting on Feb. 23, at the New York Academy of Medicine in New York City. The following papers were included in the program: "Newer Pathway of Carbohydrate Metabolism," by Paul A. Marks, M.D.; discussion by Richard J. Cross, M.D., both of the Department of Medicine, Columbia University College of Physicians and Surgeons; "Studies of Carbohydrate Metabolism in Cushing's Syndrome: Failure to Demonstrate Insensitivity to Insulin," by Maximilian Fabrykant, M.D.; discussion by Raymond S. Jackson, M.D., and Benjamin Ashe, M.D., all three of the Department of Medicine, New York University Post Graduate Medical School. George E. Anderson, M.D., Chairman, presided at the business session of the meeting which included election of new members.

THE VIRGINIA DIABETES ASSOCIATION sponsored a morning session on March 2 at the Sixth Annual Scientific Assembly of The Virginia Chapter of the American Academy of General Practice held March 2, 3 and 4, at Hot Springs, Virginia. The following lectures were given: "Diabetes in Children," by Joseph T. Beardwood, Jr., M.D., Philadelphia; "Insulin Reactions," by Robert K. Maddock, M.D.; "The Use of Various Types of Insulin," by William R. Jordan, M.D.; "Hypoglycemia," by Edward G. Davis, M.D.; and "Diabetic Acidosis," by John Franklin, M.D.

NEWS NOTES

NEW RESEARCH GRANT PROCEDURE

The U. S. Public Health Service has announced a new procedure to expedite the processing of research grants for requests not exceeding $2,000 plus indirect costs and which require support for no more than one year. Such applications will be processed on receipt and are not subject to the usual deadlines for submission prior to review.

Recommendations can be expected within one to four months from the time of application. These procedures do not apply to requests for supplements to existing grants.

Address all applications as well as requests for forms or additional information to the Division of Research Grants, National Institutes of Health, Bethesda 14, Maryland.

PERSONALS

HENRY B. MULHOLLAND, M.D., immediate past President of the American Diabetes Association, was presented recently with a distinguished service certificate by the Virginia Council on Health and Medical Care. Dr. Mulholland is Vice Chairman of the Council on Medical Service of the American Medical Association and Assistant Dean of the University of Virginia School of Medicine.

As President of the Medical Society of Virginia, Dr. Mulholland took the lead in calling together the organizations and agencies which formed the Council in 1946. He was also first Chairman from 1946 to 1950 and is now Honorary Chairman. The Council has done an outstanding job in placing physicians primarily in rural areas of Virginia, and also maintains a dental placement service.